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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:21:06 ON 21 JUL 2003

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 14:21:13 ON 21 JUL 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s alpha amylase#
FILE 'MEDLINE'
        449603 ALPHA
         20007 AMYLASE#
L1        4414 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'SCISEARCH'
        634827 ALPHA
         16041 AMYLASE#
L2        7144 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'LIFESCI'
        145006 "ALPHA"
          4245 AMYLASE#
L3        2543 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'BIOTECHDS'
         23373 ALPHA
          4734 AMYLASE#
L4        3176 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOSIS'
        590151 ALPHA
         26844 AMYLASE#
L5        9545 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'EMBASE'
        501898 "ALPHA"
         14658 AMYLASE#
L6        3251 ALPHA AMYLASE#
                 ("ALPHA"(W)AMYLASE#)

FILE 'HCAPLUS'
       1412457 ALPHA
         42336 AMYLASE#
L7       17505 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'NTIS'
         28399 ALPHA
           163 AMYLASE#
L8          60 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)
```

```
FILE 'ESBIOBASE'
        172569 ALPHA
          3676 AMYLASE#
L9        1750 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'BIOTECHNO'
        177714 ALPHA
          4041 AMYLASE#
L10       2057 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

FILE 'WPIDS'
        163176 ALPHA
          5041 AMYLASE#
L11       2082 ALPHA AMYLASE#
                 (ALPHA(W)AMYLASE#)

TOTAL FOR ALL FILES
L12      53527 ALPHA AMYLASE#

=> s l12(5a)(bacillus)
FILE 'MEDLINE'
         43281 BACILLUS
L13        460 L1 (5A)(BACILLUS)

FILE 'SCISEARCH'
         42770 BACILLUS
L14        676 L2 (5A)(BACILLUS)

FILE 'LIFESCI'
         23044 BACILLUS
L15        470 L3 (5A)(BACILLUS)

FILE 'BIOTECHDS'
         15440 BACILLUS
L16        977 L4 (5A)(BACILLUS)

FILE 'BIOSIS'
         62582 BACILLUS
L17        987 L5 (5A)(BACILLUS)

FILE 'EMBASE'
         31826 BACILLUS
L18        445 L6 (5A)(BACILLUS)

FILE 'HCAPLUS'
         74812 BACILLUS
L19       2067 L7 (5A)(BACILLUS)

FILE 'NTIS'
          1617 BACILLUS
L20          5 L8 (5A)(BACILLUS)

FILE 'ESBIOBASE'
         12225 BACILLUS
L21        209 L9 (5A)(BACILLUS)

FILE 'BIOTECHNO'
         19071 BACILLUS
L22        403 L10(5A)(BACILLUS)

FILE 'WPIDS'
         10724 BACILLUS
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```
L23          199 L11(5A)(BACILLUS)

TOTAL FOR ALL FILES
L24         6898 L12(5A)(BACILLUS)

=> s l12(5a)(muta? or variant#)
FILE 'MEDLINE'
          411830 MUTA?
           89586 VARIANT#
L25           96 L1 (5A)(MUTA? OR VARIANT#)

FILE 'SCISEARCH'
          389517 MUTA?
           94316 VARIANT#
L26          114 L2 (5A)(MUTA? OR VARIANT#)

FILE 'LIFESCI'
          185562 MUTA?
           30766 VARIANT#
L27           89 L3 (5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
           34758 MUTA?
           10461 VARIANT#
L28          159 L4 (5A)(MUTA? OR VARIANT#)

FILE 'BIOSIS'
          458979 MUTA?
           94375 VARIANT#
L29          225 L5 (5A)(MUTA? OR VARIANT#)

FILE 'EMBASE'
          334971 MUTA?
           77629 VARIANT#
L30           86 L6 (5A)(MUTA? OR VARIANT#)

FILE 'HCAPLUS'
          421301 MUTA?
           87198 VARIANT#
L31          360 L7 (5A)(MUTA? OR VARIANT#)

FILE 'NTIS'
            9404 MUTA?
            4394 VARIANT#
L32            1 L8 (5A)(MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
          190505 MUTA?
           32550 VARIANT#
L33           52 L9 (5A)(MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
          227060 MUTA?
           38799 VARIANT#
L34           70 L10(5A)(MUTA? OR VARIANT#)

FILE 'WPIDS'
           21755 MUTA?
           20900 VARIANT#
L35           65 L11(5A)(MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L36         1317 L12(5A)(MUTA? OR VARIANT#)
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```
=> s l24 and l36
FILE 'MEDLINE'
L37         31 L13 AND L25

FILE 'SCISEARCH'
L38         35 L14 AND L26

FILE 'LIFESCI'
L39         31 L15 AND L27

FILE 'BIOTECHDS'
L40         86 L16 AND L28

FILE 'BIOSIS'
L41         56 L17 AND L29

FILE 'EMBASE'
L42         31 L18 AND L30

FILE 'HCAPLUS'
L43        155 L19 AND L31

FILE 'NTIS'
L44          0 L20 AND L32

FILE 'ESBIOBASE'
L45          8 L21 AND L33

FILE 'BIOTECHNO'
L46         19 L22 AND L34

FILE 'WPIDS'
L47         32 L23 AND L35

TOTAL FOR ALL FILES
L48        484 L24 AND L36

=> s l48 not 1997-1999/py
FILE 'MEDLINE'
       1334163 1997-1999/PY
L49         28 L37 NOT 1997-1999/PY

FILE 'SCISEARCH'
       2862496 1997-1999/PY
L50         31 L38 NOT 1997-1999/PY

FILE 'LIFESCI'
        337241 1997-1999/PY
L51         28 L39 NOT 1997-1999/PY

FILE 'BIOTECHDS'
         41048 1997-1999/PY
L52         72 L40 NOT 1997-1999/PY

FILE 'BIOSIS'
       1680857 1997-1999/PY
L53         50 L41 NOT 1997-1999/PY

FILE 'EMBASE'
       1252969 1997-1999/PY
L54         27 L42 NOT 1997-1999/PY

FILE 'HCAPLUS'
       2535747 1997-1999/PY
```

```
L55         121 L43 NOT 1997-1999/PY

FILE 'NTIS'
          85501 1997-1999/PY
L56           0 L44 NOT 1997-1999/PY

FILE 'ESBIOBASE'
         831730 1997-1999/PY
L57           4 L45 NOT 1997-1999/PY

FILE 'BIOTECHNO'
         338670 1997-1999/PY
L58          16 L46 NOT 1997-1999/PY

FILE 'WPIDS'
        2351774 1997-1999/PY
L59          15 L47 NOT 1997-1999/PY

TOTAL FOR ALL FILES
L60         392 L48 NOT 1997-1999/PY

=> s l60 not 2000-2003/py
FILE 'MEDLINE'
        1799406 2000-2003/PY
L61          27 L49 NOT 2000-2003/PY

FILE 'SCISEARCH'
        3395666 2000-2003/PY
L62          30 L50 NOT 2000-2003/PY

FILE 'LIFESCI'
         336333 2000-2003/PY
L63          27 L51 NOT 2000-2003/PY

FILE 'BIOTECHDS'
          64188 2000-2003/PY
L64          58 L52 NOT 2000-2003/PY

FILE 'BIOSIS'
        1845366 2000-2003/PY
L65          46 L53 NOT 2000-2003/PY

FILE 'EMBASE'
        1539979 2000-2003/PY
L66          27 L54 NOT 2000-2003/PY

FILE 'HCAPLUS'
        3406755 2000-2003/PY
L67          96 L55 NOT 2000-2003/PY

FILE 'NTIS'
          56051 2000-2003/PY
L68           0 L56 NOT 2000-2003/PY

FILE 'ESBIOBASE'
         985170 2000-2003/PY
L69           4 L57 NOT 2000-2003/PY

FILE 'BIOTECHNO'
         401781 2000-2003/PY
L70          16 L58 NOT 2000-2003/PY

FILE 'WPIDS'
        3060663 2000-2003/PY
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L71 5 L59 NOT 2000-2003/PY

TOTAL FOR ALL FILES
L72 336 L60 NOT 2000-2003/PY

=> dup rem l72
PROCESSING COMPLETED FOR L72
L73 166 DUP REM L72 (170 DUPLICATES REMOVED)

=> d tot

L73 ANSWER 1 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Laundry detergent containing a **mutant alpha-amylase**;
Bacillus licheniformis recombinant enzyme production by enzyme engineering, for application as a laundry surfactant
AU Barnett C C; Boyer S G; Mitchinson C; Power S D
AN 1997-00549 BIOTECHDS
PI WO 9630481 3 Oct 1996

L73 ANSWER 2 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Improved bleach-containing cleaning composition;
granular bleach-containing granular surfactant containing **Bacillus** licheniformis **mutant alpha-amylase** and **Bacillus** amyloliquefaciens **mutant** protease
AU Barnett C C; Mitchinson C; Power S D
AN 1996-06019 BIOTECHDS
PI WO 9605295 22 Feb 1996

L73 ANSWER 3 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Acid-stable and thermostable alpha-amylase gene;
characterization from Bacillus licheniformis; enzyme engineering
AN 1997-01693 BIOTECHDS
PI JP 08289788 5 Nov 1996

L73 ANSWER 4 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Purification and characterization of a truncated **Bacillus** subtilis .**alpha**.-**amylase** produced by Escherichia coli
SO Applied Microbiology and Biotechnology (1996), 44(6), 746-52
CODEN: AMBIDG; ISSN: 0175-7598
AU Marco, J. L.; Bataus, L. A.; Valencia. F. F.; Ulhoa, C. J.; Astolfi-Filho, S.; Felix, C. R.
AN 1996:206488 HCAPLUS
DN 124:336337

L73 ANSWER 5 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Structure-function studies of two polysaccharide-degrading enzymes: **Bacillus** stearothermophilus .**alpha**.-**amylase** and Trichoderma reesei cellobiohydrolase II
SO VTT Publications (1996), 277, 143pp
CODEN: VTTPEY; ISSN: 1235-0621
AU Koivula, Anu
AN 1996:749573 HCAPLUS
DN 126:28530

L73 ANSWER 6 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Instability of .alpha.-amylase production and morphological variation in continuous culture of Bacillus amyloliquefaciens is associated with plasmid loss
SO Process Biochemistry (Oxford) (1996), Volume Date 1997, 32(1), 51-59
CODEN: PBCHE5; ISSN: 1359-5113
AU Hillier, P.; Wase, D. A. J.; Emery, A. N.; Solomons, G. L.
AN 1996:695106 HCAPLUS

DN 125:326485

L73 ANSWER 7 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Hyperthermostable mutants of Bacillus licheniformis: thermodynamic studies and structural interpretation
SO Perspectives on Protein Engineering '96, [International Conference], 5th, Montpellier, Fr., 1996 (1996), Paper No. 7, 9 pp.. Editor(s): Geisow, Michael J. Publisher: BIODIGM, Bingham, UK.
CODEN: 64HIAR
AU Declerck, Nathalie; Gaillardin, Claude; Machius, Mischa; Wiegand, Georg; Huber, Robert
AN 1997:287296 HCAPLUS
DN 126:314064

L73 ANSWER 8 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI **Mutant** B. licheniformis **alpha-amylase** enzymes;
Bacillus licheniformis mutant thermostable enzyme production; application in starch degradation, textile or paper desizing, brewing industry and as household surfactant
AU van der Laan J M; Aehle W
AN 1996-03039 BIOTECHDS
PI WO 9535382 28 Dec 1995

L73 ANSWER 9 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI New **alpha-amylase variants**;
Bacillus liquefaciens **alpha-amylase** enzyme engineering for improved thermostability, pH stability, etc.; application in surfactant composition to improve washing performance
AU Bisgard-Frantzen H; Borchert T V; Svendsen A; Thellersen M; van der Zee P
AN 1995-07973 BIOTECHDS
PI WO 9510603 20 Apr 1995

L73 ANSWER 10 OF 166 MEDLINE on STN DUPLICATE 1
TI Hyperthermostable **mutants** of **Bacillus** licheniformis **alpha-amylase**: multiple amino acid replacements and molecular modelling.
SO PROTEIN ENGINEERING, (1995 Oct) 8 (10) 1029-37.
Journal code: 8801484. ISSN: 0269-2139.
AU Declerck N; Joyet P; Trosset J Y; Garnier J; Gaillardin C
AN 96367070 MEDLINE

L73 ANSWER 11 OF 166 MEDLINE on STN DUPLICATE 2
TI Effects of signal peptide **mutations** on processing of **Bacillus** stearothermophilus **alpha-amylase** in Escherichia coli.
SO MICROBIOLOGY, (1995 Mar) 141 (Pt 3) 649-54.
Journal code: 9430468. ISSN: 1350-0872.
AU Suominen I; Meyer P; Tilgmann C; Glumoff T; Glumoff V; Kapyla J; Mantsala P
AN 95227363 MEDLINE

L73 ANSWER 12 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 3
TI COLONY SWITCHING IN AN **ALPHA-AMYLASE**-PRODUCING STRAIN OF **BACILLUS**-SUBTILIS
SO JOURNAL OF INDUSTRIAL MICROBIOLOGY, (AUG 1995) Vol. 15, No. 2, pp. 112-115.
ISSN: 0169-4146.
AU RODRIGUEZ H (Reprint)
AN 95:642643 SCISEARCH

L73 ANSWER 13 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Studies on alkaline amylase from alkaliphilic Bacillus megaterium. II. Selection of high producing strain and conditions for enzyme production;

improved **alpha-amylase** production by **mutant** strain
SO Ind.Microbiol.; (1995) 25, 1, 13-16
CODEN: GOWEEK
AU Shiru J; Shuxin Z; Jinghua L; Xiaoli C
AN 1995-07944 BIOTECHDS

L73 ANSWER 14 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Bacillus licheniformis, **Bacillus** stearothermophilus and **Bacillus** amyloliquefaciens **alpha-amylase** enzyme engineering by site-directed **mutagenesis**;
DNA sequence; application in a surfactant or a starch liquefaction composition
AN 1994-13784 BIOTECHDS
PI WO 9418314 18 Aug 1994

L73 ANSWER 15 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Lipase and **alpha-amylase variant** stabilized against peroxidase system;
Humicola lanuginosa or Bacillus licheniformis enzyme stabilization by enzyme engineering for use in a surfactant composition
AN 1994-11299 BIOTECHDS
PI WO 9414951 7 Jul 1994

L73 ANSWER 16 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI **Mutant alpha-amylase** from **Bacillus** sp. use as surfactant, dish washing agent and liquefaction agent;
Bacillus or Aspergillus spp. thermostable enzyme with increased thermostability and activity at low pH produced by enzyme engineering
AN 1994-04189 BIOTECHDS
PI WO 9402597 3 Feb 1994

L73 ANSWER 17 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Saccharomycopsis fibuligera **alpha-amylase** or **Bacillus** macerans cyclomaltodextrin-glucanotransferase **mutant** preparation;
enzyme engineering for improved production of oligosaccharide
AN 1995-00657 BIOTECHDS
PI JP 06253836 13 Sep 1994

L73 ANSWER 18 OF 166 MEDLINE on STN DUPLICATE 4
TI Site-directed mutagenesis reveals critical importance of the catalytic site in the binding of alpha-amylase by wheat proteinaceous inhibitor.
SO BIOCHEMISTRY, (1994 Jun 28) 33 (25) 7925-30.
Journal code: 0370623. ISSN: 0006-2960.
AU Takase K
AN 94281224 MEDLINE

L73 ANSWER 19 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 5
TI C-TERMINAL TRUNCATIONS OF A THERMOSTABLE **BACILLUS** -STEAROTHERMOPHILUS **ALPHA-AMYLASE**
SO PROTEIN ENGINEERING, (OCT 1994) Vol. 7, No. 10, pp. 1255-1259.
ISSN: 0269-2139.
AU VIHINEN M (Reprint); PELTONEN T; IITIA A; SUOMINEN I; MANTSALA P
AN 94:668008 SCISEARCH

L73 ANSWER 20 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 6
TI CHANGES IN OPTIMUM PH AND THERMOSTABILITY OF **ALPHA-AMYLASE** FROM **BACILLUS**-LICHENIFORMIS BY SITE-DIRECTED MUTAGENESIS OF HIS-235 AND ASP-328
SO BULLETIN OF THE KOREAN CHEMICAL SOCIETY, (20 OCT 1994) Vol. 15, No. 10, pp. 832-835.
ISSN: 0253-2964.
AU KIM M S (Reprint); LEE S K; JUNG H S; YANG C H

AN 94:725048 SCISEARCH

L73 ANSWER 21 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Experimental study on the technological conditions of .**alpha**.-**amylase** fermentation with **Bacillus mutant**
SO Zhejiang Gongxueyuan Xuebao (1994), (2), 56-61
CODEN: ZGXUEM; ISSN: 1000-209X
AU Zheng, Yuguo; Cao, Xiaoru; Lu, Jianwei; Li, Xiaoqin
AN 1994:653778 HCAPLUS
DN 121:253778

L73 ANSWER 22 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Selection and breeding of a high productivity strain of .**alpha**.-**amylase** from the multi-resistant **mutants** of Bacillus
SO Wuxi Qinggongye Xueyuan Xuebao (1994), 13(1), 21-6
CODEN: WQXUEP; ISSN: 1001-7453
AU Wang, Zhao; Ji, Qirong
AN 1994:678946 HCAPLUS
DN 121:278946

L73 ANSWER 23 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI **Mutant Bacillus** licheniformis **alpha-amylase** promoter, vector and DNA sequence;
application in alpha-amylase, cyclomaltodextrin-glucanotransferase, glucosyltransferase and protease production
AN 1993-09548 BIOTECHDS
PI WO 9310249 27 May 1993

L73 ANSWER 24 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI New **mutant Bacillus** licheniformis **alpha-amylase** signal peptide sequence;
to give reduced processing ambiguity during e.g. human recombinant interleukin-3 protein secretion
AN 1994-01346 BIOTECHDS
PI EP 572088 1 Dec 1993

L73 ANSWER 25 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Gene expressing in **Bacillus** licheniformis using especially .**alpha**.-**amylase** promoter **variant**
SO PCT Int. Appl., 63 pp.
CODEN: PIXXD2
IN Joergensen, Steen Troels; Joergensen, Per Linaa
AN 1993:553380 HCAPLUS
DN 119:153380

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9310248	A1	19930527	WO 1992-DK337	19921113
W: FI, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
JP 07503363	T2	19950413	JP 1993-508898	19921113
EP 672154	A1	19950920	EP 1992-923721	19921113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
FI 9402227	A	19940513	FI 1994-2227	19940513

L73 ANSWER 26 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Construction of a model secretion system for oral streptococci
SO Infection and Immunity (1993), 61(9), 3745-55
CODEN: INFIBR; ISSN: 0019-9567
AU Shiroza, Teruaki; Kuramitsu, Howard K.
AN 1993:642285 HCAPLUS
DN 119:242285

L73 ANSWER 27 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Efficient production of **Bacillus** stearothermophilus

alpha-amylase in **Bacillus** brevis by altering its signal peptide;
effect of signal peptide sequence mutagenesis or replacement of protein secretion
SO Biosci.Biotechnol.Biochem.; (1993) 57, 8, 1384-86
CODEN: BBBIEJ
AU Yamaguchi K; Ueda M; Udaka S
AN 1993-12419 BIOTECHDS

L73 ANSWER 28 OF 166 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V. on STN DUPLICATE
AN 1994016204 ESBIOBASE
TI Crystallization and preliminary X-ray studies of wild type and catalytic-site **mutant alpha-amylase** from **Bacillus** subtilis
AU Mizuno H.; Morimoto Y.; Tsukihara T.; Matsumoto T.; Takase K.
CS H. Mizuno, Japan.
SO Journal of Molecular Biology, (1993), 234/4 (1283-1293)
CODEN: JMOBAK ISSN: 0022-2836
DT Journal; Article
LA English
SL English

L73 ANSWER 29 OF 166 MEDLINE on STN DUPLICATE 8
TI Crystallization and preliminary X-ray studies of wild type and catalytic-site **mutant alpha-amylase** from **Bacillus** subtilis.
SO JOURNAL OF MOLECULAR BIOLOGY, (1993 Dec 20) 234 (4) 1282-3.
Journal code: 2985088R. ISSN: 0022-2836.
AU Mizuno H; Morimoto Y; Tsukihara T; Matsumoto T; Takase K
AN 94087744 MEDLINE

L73 ANSWER 30 OF 166 MEDLINE on STN DUPLICATE 9
TI Effect of mutation of an amino acid residue near the catalytic site on the activity of **Bacillus** stearothermophilus **alpha-amylase**.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1993 Feb 1) 211 (3) 899-902.
Journal code: 0107600. ISSN: 0014-2956.
AU Takase K
AN 93170327 MEDLINE

L73 ANSWER 31 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 10
TI Transformation of **Bacillus** subtilis in **alpha-amylase** production by **mutant** DNA.
SO Journal of the Chinese Agricultural Chemical Society, (1993) Vol. 31, No. 4, pp. 454-465.
ISSN: 0578-1736.
AU Wang, Mei-Jen; Chou, Cheng-Chun; Yu, Roch-Chui
AN 1994:269713 BIOSIS

L73 ANSWER 32 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Purification and characterization of a thermostable **alpha-amylase** from **Bacillus** licheniformis;
enzyme isolation and properties
SO J.Biotechnol.; (1993) 28, 2-3, 277-89
CODEN: JBITD4
AU Ivanova V N; Dobreva E P; Emanuilova E I
AN 1993-07233 BIOTECHDS

L73 ANSWER 33 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Stability of industrial enzymes;
enzyme stabilization by chemical modification or enzyme engineering (conference paper)

SO Stud.Org.Chem.; (1993) 47, 111-31
CODEN: 9999T
AU Misset O
AN 1994-05917 BIOTECHDS

L73 ANSWER 34 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Nucleotide sequence of **Bacillus** stearothermophilus **alpha-amylase** gene and its high expression;
cloning and DNA sequence
SO Ind.Microbiol.; (1993) 23, 2, 1-7
CODEN: GOWEEK
AU Xu Y; Wang X; He C; Wu C; Ren D
AN 1994-02920 BIOTECHDS

L73 ANSWER 35 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI New thermostable forms of **Bacillus** licheniformis **alpha -amylase**;
enzyme engineering by specific amino acid substitutions at positions 133 and or 209, for simultaneous gelation and liquefaction of starch, e.g. in brewing
AN 1993-03609 BIOTECHDS
PI FR 2676456 20 Nov 1992

L73 ANSWER 36 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI New thermostable **alpha-amylase** from **Bacillus** licheniformis;
obtained by enzyme engineering and useful in paper-making, brewing etc. for starch liquefaction
AN 1992-07694 BIOTECHDS
PI FR 2665178 31 Jan 1992

L73 ANSWER 37 OF 166 MEDLINE on STN DUPLICATE 14
TI Hyperthermostable **variants** of a highly thermostable **alpha-amylase**.
SO BIO/TECHNOLOGY, (1992 Dec) 10 (12) 1579-83.
Journal code: 8309273. ISSN: 0733-222X.
AU Joyet P; Declerck N; Gaillardin C
AN 93168398 MEDLINE

L73 ANSWER 38 OF 166 MEDLINE on STN DUPLICATE 15
TI Site-directed mutagenesis of active site residues in **Bacillus** subtilis **alpha-amylase**.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1992 Apr 17) 1120 (3) 281-8.
Journal code: 0217513. ISSN: 0006-3002.
AU Takase K; Matsumoto T; Mizuno H; Yamane K
AN 92247808 MEDLINE

L73 ANSWER 39 OF 166 MEDLINE on STN DUPLICATE 16
TI Interaction of catalytic-site **mutants** of **Bacillus** subtilis **alpha-amylase** with substrates and acarbose.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1992 Aug 21) 1122 (3) 278-82.
Journal code: 0217513. ISSN: 0006-3002.
AU Takase K
AN 92369111 MEDLINE

L73 ANSWER 40 OF 166 MEDLINE on STN DUPLICATE 17
TI Functional relationships between cyclodextrin glucanotransferase from an alkalophilic **Bacillus** and **alpha-amylases**.
Site-directed **mutagenesis** of the conserved two Asp and one Glu residues.
SO FEBS LETTERS, (1992 Jan 13) 296 (1) 37-40.
Journal code: 0155157. ISSN: 0014-5793.
AU Nakamura A; Haga K; Ogawa S; Kuwano K; Kimura K; Yamane K
AN 92111781 MEDLINE

L73 ANSWER 41 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Comparison of microbial amylase production by various mutants derived from Bacillus amyloliquefaciens
SO Gongye Weishengwu (1992), 22(2), 26-9
CODEN: GOWEEK; ISSN: 1001-6678
AU Hu, Xuezhi; Hou, Qinfang; Ling, Chen
AN 1992:549367 HCAPLUS
DN 117:149367

L73 ANSWER 42 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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Bacillus amyloliquefaciens **alpha-amylase** **mutant** expression in e.g. Escherichia coli, Bacillus, Aspergillus spp.; bread improver with reduced thermostability during baking; DNA sequence
AN 1991-04156 BIOTECHDS
PI EP 409299 23 Jan 1991

L73 ANSWER 43 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 18
TI Random point mutation analysis of the signal peptide cleavage area of **Bacillus** stearothermophilus **alpha -amylase**.
SO AGRIC. BIOL. CHEM., (1991) vol. 55, no. 11, pp. 2875-2876.
AU Yamaguchi, K.; Ueda, M.; Kawanishi, G.; Udaka, S.
AN 93:1248 LIFESCI

L73 ANSWER 44 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI A new pleiotropic mutation affecting purine metabolism, sporulation and biosynthesis of exoenzymes in Bacillus subtilis
SO Genetika (Moscow) (1991), 27(6), 983-90
CODEN: GNKAA5; ISSN: 0016-6758
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AN 1992:16397 HCAPLUS
DN 116:16397

L73 ANSWER 45 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Isolation of .**alpha**.-**amylase** hyperproducing strain HG4 from **Bacillus** sp. and some properties of the enzyme
SO Sanop Misaengmul Hakhoechi (1991), 19(5), 464-9
CODEN: SMHAEH; ISSN: 0257-2389
AU Kim, Moo Sung; O, Pyong Su
AN 1993:537438 HCAPLUS
DN 119:137438

L73 ANSWER 46 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 19
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SO J. FERMENT. BIOENG., (1991) vol. 71, no. 6, pp. 397-402.
ISSN: 0922-338X.
AU Ariga, O.; Andoh, Y.; Fujishita, Y.; Watari, T.; Sano, Y.
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L73 ANSWER 47 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI PRODUCTION OF THERMOPHILIC ALPHA-AMYLASE USING IMMOBILIZED TRANSFORMED ESCHERICHIA-COLI BY ADDITION OF GLYCINE
SO JOURNAL OF FERMENTATION AND BIOENGINEERING, (1991) Vol. 71, No. 6, pp. 397-402.
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AN 91:390037 SCISEARCH

L73 ANSWER 48 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
TI STUDIES ON THE SCREENING OF THERMOSTABLE ALPHA AMYLASE-PRODUCING STRAINS.
SO ACTA MICROBIOL SIN, (1991) 31 (4), 268-273.
CODEN: WSHPA8. ISSN: 0001-6209.

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AN 1992:71266 BIOSIS

L73 ANSWER 49 OF 166 LIFESCI COPYRIGHT 2003 CSA on STN
TI Studies on the screening of thermostable alpha -amylase-producing strains
SO ACTA MICROBIOL. SIN., (1991) vol. 31, no. 4, pp. 267-273.
ISSN: 0001-6209.
AU Xuezhi, Hu; Chen, Ling; Qinfang, Hou; Zhaozu, Lei
AN 94:37980 LIFESCI

L73 ANSWER 50 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Effect of glucose and citrate on **alpha-amylase** production in **Bacillus** licheniformis;
effect of glucose, citric acid culture medium C-source
SO J.Basic Microbiol.; (1991) 31, 3, 217-22
CODEN: JBMIEQ
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AN 1991-10760 BIOTECHDS

L73 ANSWER 51 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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AU TONKOVA A (Reprint)
AN 91:402521 SCISEARCH

L73 ANSWER 52 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Site-specific mutagenesis of **Bacillus** stearothermophilus and **Bacillus** licheniformis **alpha-amylase** hybrids;
enzyme engineering (conference abstract)
SO J.Cell.Biochem.; (1991) Suppl.15G, 214
CODEN: JCEBD5
AU Requadt C; Mainzer S; Stephens-Jarnagin A; Power S D
AN 1991-08324 BIOTECHDS

L73 ANSWER 53 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Isolation of thermostable .**alpha**.-**amylase** hyperproducing **Bacillus** sp. No. 32H417 and some properties of the enzyme
SO Sanop Misaengmul Hakhoechi (1991), 19(2), 122-7
CODEN: SMHAEH; ISSN: 0257-2389
AU Kim, Moo Sung; O, Pyong Su
AN 1993:556049 HCAPLUS
DN 119:156049

L73 ANSWER 54 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Jpn. Kokai Tokkyo Koho, 11 pp.
CODEN: JKXXAF
IN Imanaka, Tadayuki; Sai, Kiken; Ken, Masuo
AN 1991:201125 HCAPLUS
DN 114:201125

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PI	JP 02222687	A2	19900905	JP 1989-43561	19890225

L73 ANSWER 55 OF 166 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
TI Mutant Bacillus brevis - is used to produce large amt. of protein and human salivary alpha-amylase, etc..
PI JP 02257876 A 19901018 (199048)*

L73 ANSWER 56 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Use of amber suppressors to investigate the thermostability of

Bacillus licheniformis **alpha-amylase**: amino acid replacements at 6 histidine residues reveal a critical position at His-133;
thermostable enzyme gene site-directed mutagenesis using amber suppressor mutation; protein engineering
SO J.Biol.Chem.; (1990) 265, 26, 15481-88
CODEN: JBCHA3
AU Declerck N; Joyet P; Gaillardin C; Masson J M
AN 1990-13821 BIOTECHDS

L73 ANSWER 57 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Site-directed mutagenesis of a catabolite repression operator sequence in Bacillus subtilis
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CODEN: PNASA6; ISSN: 0027-8424
AU Weickert, Michael J.; Chambliss, Glenn H.
AN 1990:527370 HCAPLUS
DN 113:127370

L73 ANSWER 58 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Processing of an amino-terminally extended thermostable .**alpha**.-**amylase** by **Bacillus** subtilis alkaline protease
SO Journal of Biochemistry (Tokyo, Japan) (1990), 108(6), 954-9
CODEN: JOBIAO; ISSN: 0021-924X
AU Itoh, Yoshiki; Sumi, Masahiro; Nakamura, Kouji; Yamane, Kunio
AN 1991:38427 HCAPLUS
DN 114:38427

L73 ANSWER 59 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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CODEN: PRENE9. ISSN: 0269-2139.
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AN 1990:403351 BIOSIS

L73 ANSWER 60 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
TI SITE-DIRECTED **MUTAGENESIS** OF **BACILLUS**-SUBTILIS **ALPHA AMYLASE** SHOWS GLU208 AS A CATALYTIC RESIDUE.
SO MEETING ON PROTEIN ENGINEERING, 1989, KOBE, JAPAN, AUGUST 20-25, 1989. PROTEIN ENG. (1990) 3 (4), 351-352.
CODEN: PRENE9. ISSN: 0269-2139.
AU TAKASE K; MATSUMOTO T; MIZUNO H; YAMANE K
AN 1990:403348 BIOSIS

L73 ANSWER 61 OF 166 MEDLINE on STN DUPLICATE 21
TI Efficient production of human **alpha-amylase** by a **Bacillus** brevis **mutant**.
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1990 Dec) 34 (3) 297-302.
Journal code: 8406612. ISSN: 0175-7598.
AU Konishi H; Sato T; Yamagata H; Udaka S
AN 91254745 MEDLINE

L73 ANSWER 62 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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AU KONISHI H; SATO T; YAMAGATA H (Reprint); UDAKA S
AN 91:34627 SCISEARCH

L73 ANSWER 63 OF 166 MEDLINE on STN DUPLICATE 22
TI Site-directed **mutagenesis** of a thermostable **alpha-amylase** from **Bacillus** stearothermophilus: putative role of three conserved residues.
SO JOURNAL OF BIOCHEMISTRY, (1990 Feb) 107 (2) 267-72.
Journal code: 0376600. ISSN: 0021-924X.
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AN 90299864 MEDLINE

L73 ANSWER 64 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI SITE-DIRECTED **MUTAGENESIS** OF A THERMOSTABLE **ALPHA-AMYLASE** FROM **BACILLUS**-STEAROTHERMOPHILUS - PUTATIVE ROLE OF 3 CONSERVED RESIDUES
SO JOURNAL OF BIOCHEMISTRY, (1990) Vol. 107, No. 2, pp. 267-272.
AU VIHINEN M (Reprint); OLLIKKA P; NISKANEN J; MEYER P; SUOMINEN I; KARP M; HOLM L; KNOWLES J; MANTSALA P
AN 90:90466 SCISEARCH

L73 ANSWER 65 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Recent results of amylolytic enzymes research; Aspergillus niger and **Bacillus** licheniformis strain improvement for thermostable **alpha-amylase** and glucoamylase production; UV-irradiation **mutagenesis**, selection, transformation or protoplast fusion
SO Acta Aliment.Acad.Sci.Hung.; (1990) 19, 2, 210-11
CODEN: AAASCO
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L73 ANSWER 66 OF 166 MEDLINE on STN DUPLICATE 23
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AN 90231977 MEDLINE

L73 ANSWER 67 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: FMLED7; ISSN: 0378-1097
AU Maznitsa, I. I.; Nudler, A. A.; Burd, G. I.
AN 1991:20849 HCAPLUS
DN 114:20849

L73 ANSWER 68 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 24
TI HIGH PRODUCTION STRAIN OF **ALPHA AMYLASE** FROM TUNICAMYCIN-RESISTANT **MUTANTS** OF **BACILLUS**-SUBTILIS BF7658.
SO ACTA MICROBIOL SIN, (1990) 30 (1), 83-85.
CODEN: WSHPA8. ISSN: 0001-6209.
AU JIA S; GUO X
AN 1991:25912 BIOSIS

L73 ANSWER 69 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Selection of the lysine-producing strains in starch medium
SO Shipin Yu Fajiao Gongye (1990), (1), 57-62
CODEN: SPYYDO; ISSN: 0253-990X
AU Jia, Shinu; Zhao, Shuqin; Ye, Wen
AN 1990:438799 HCAPLUS
DN 113:38799

L73 ANSWER 70 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Amylase and protease production by Bacillus subtilis: studies on improvement of strain and medium
SO Journal of Food Science and Technology (1990), 27(1), 4-6
CODEN: JFSTAB; ISSN: 0022-1155
AU Kadrekar, S. N.; Ramasarma, G. B.
AN 1990:457391 HCAPLUS
DN 113:57391

L73 ANSWER 71 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STNDUPLICATE 25
TI Manufacture of thermostable alpha-amylases with superproductive microorganisms at high temperatures
SO Eur. Pat. Appl., 9 pp.
CODEN: EPXXDW
IN Perez Pascual, Miguel Antonio; Del Castillo Roman, Francisco; Martinez Reif, Pilar; Murillo Aranjo, Francisco Jose; Ruiz Vazquez, Rosa Maria; Balsalobre Balibrea, Josefa Maria
AN 1990:53755 HCAPLUS
DN 112:53755

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 320685	A1	19890621	EP 1988-119794	19881128
EP 320685	B1	19931013		
R: BE, DE, FR, GB, IT, NL				
ES 2007758	A6	19890701	ES 1987-3510	19871207
FI 8705567	A	19890608	FI 1987-5567	19871217
FI 89077	B	19930430		
FI 89077	C	19930810		
DK 8806801	A	19890608	DK 1988-6801	19881206

L73 ANSWER 72 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Amino acid residues stabilizing a **Bacillus alpha-amylase** against irreversible thermoinactivation; enzyme engineering by site directed mutagenesis and chimeric gene construction with **Bacillus** licheniformis and **Bacillus** amyloliquefaciens **alpha-amylase** gene
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CODEN: JBCHA3
AU Suzuki Y; Ito N; Yuuki T; *Yamagata H; Udaka S
AN 1990-00500 BIOTECHDS

L73 ANSWER 73 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
TI CIS SEQUENCES INVOLVED IN MODULATING EXPRESSION OF BACILLUS-LICHENIFORMIS AMY-L IN BACILLUS-SUBTILIS EFFECT OF SPORULATION MUTATIONS AND CATABOLITE REPRESSION RESISTANCE MUTATIONS ON EXPRESSION.
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CODEN: JOBAAY. ISSN: 0021-9193.
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L73 ANSWER 74 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 26
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SO KOREAN J APPL MICROBIOL BIOENG, (1989) 17 (5), 412-420.
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AN 1990:130964 BIOSIS

L73 ANSWER 75 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Regulation of **Bacillus** subtilis **alpha-amylase** synthesis;

glucose catabolite repression elimination by cis, trans mutation (conference abstract)
SO Folia Microbiol.; (1989) 34, 5, 364
CODEN: FOMIAZ
AU Chambliss G H; Nicholson W L; Weickert M J
AN 1990-07223 BIOTECHDS

L73 ANSWER 76 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Predominant growth of .**alpha**.-**amylase** regulation **mutant** in continuous culture of Bacillus caldolyticus
SO Journal of Fermentation and Bioengineering (1989), 67(3), 176-81
CODEN: JFBIEX; ISSN: 0922-338X
AU Cheng, Chu Yuan; Yabe, Isamu; Toda, Kiyoshi
AN 1989:191188 HCAPLUS
DN 110:191188

L73 ANSWER 77 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 27
TI Selective growth of a mutant in continuous culture of **Bacillus** caldolyticus for production of **alpha** -**amylase**.
SO APPL. MICROBIOL. BIOTECHNOL., (1989) vol. 30, no. 2, pp. 125-129.
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AN 89:32088 LIFESCI

L73 ANSWER 78 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Frequency distribution of .alpha.-amylase productivity among cells growing in continuous culture of Bacillus caldolyticus strain SP
SO Hakko Kogaku Kaishi (1989), 67(2), 91-7
CODEN: HKOKDE; ISSN: 0385-6151
AU Cheng, Chuyuan; Yabe, Isamu; Toda, Kiyoshi
AN 1989:191193 HCAPLUS
DN 110:191193

L73 ANSWER 79 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: CITAC7; ISSN: 0100-350X
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AN 1990:137440 HCAPLUS
DN 112:137440

L73 ANSWER 80 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 28
TI ISOLATION OF A STABLE AND HIGH YIELDING **ALPHA-AMYLASE MUTANT** OF **BACILLUS**-SUBTILIS
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AN 89:428064 SCISEARCH

L73 ANSWER 81 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Gekkan Fudo Kemikaru (1989), 5(7), 31-7
CODEN: GFKEEX; ISSN: 0911-2286
AU Yamane, Kunio
AN 1989:547515 HCAPLUS
DN 111:147515

L73 ANSWER 82 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Methods for selection of .**alpha**.-**amylase** superproducing **mutants**
SO Weishengwuxue Tongbao (1989), 16(1), 3-5
CODEN: WSWPDI; ISSN: 0253-2654
AU Men, Dapeng; Jia, Shifang; Guo, Xinghua

AN 1989:495531 HCAPLUS
DN 111:95531

L73 ANSWER 83 OF 166 MEDLINE on STN DUPLICATE 29
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Journal code: 0125036. ISSN: 0026-8925.
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AN 89281475 MEDLINE

L73 ANSWER 84 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Eur. Pat. Appl., 16 pp.
CODEN: EPXXDW
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AN 1989:187342 HCAPLUS
DN 110:187342

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 285123	A2	19881005	EP 1988-105163	19880330
EP 285123	A3	19890201		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FI 8801530	A	19881004	FI 1988-1530	19880331
JP 01020089	A2	19890124	JP 1988-81159	19880401

L73 ANSWER 85 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Complete nucleotide sequence of an **alpha-amylase** gene from **Bacillus** subtilis 2633, an **alpha-amylase** extrahyperproducing strain;
comparison with **alpha-amylase** genes in other **Bacillus** subtilis strains
SO Nucleic Acids Res.; (1988) 16, 14B, 7178
CODEN: NARHAD
AU Emori M; Maruo B
AN 1988-10076 BIOTECHDS

L73 ANSWER 86 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Agricultural and Biological Chemistry (1988), 52(9), 2365-6
CODEN: ABCHA6; ISSN: 0002-1369
AU Yoshigi, Naohiro; Kamimura, Minoru
AN 1988:608090 HCAPLUS
DN 109:208090

L73 ANSWER 87 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 30
TI Mutants of Bacillus subtilis defective in protein export.
SO J. GEN. MICROBIOL., (1988) vol. 134, no. 8, pp. 2333-2344.
AU Kontinen, V.P.; Sarvas, M.
AN 88:40041 LIFESCI

L73 ANSWER 88 OF 166 MEDLINE on STN DUPLICATE 31
TI [Two-codon **mutagenesis** of **alpha-amylase** gene of **Bacillus** amyloliquefaciens].
Dvukhkodonnyi mutagenez gena alpha-amilazy Bacillus amyloliquefaciens.
SO MOLEKULIARNAIA BIOLOGIIA, (1988 Sep-Oct) 22 (5) 1265-71.
Journal code: 0105454. ISSN: 0026-8984.
AU Smirnova N A; Sorokin A V; Laptev D A; Veiko V P; Kozlov Iu I
AN 89127179 MEDLINE

L73 ANSWER 89 OF 166 MEDLINE on STN DUPLICATE 32
TI [**Mutations** in the **alpha-amylase** gene of **Bacillus** amyloliquefaciens, leading to a decrease in the temperature of protein inactivation].
Mutatsii v gene alpha-amilazy Bacillus amyloliquefaciens, privodiashchie k snizheniiu temperatury inaktivatsii belka.
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Journal code: 0105454. ISSN: 0026-8984.
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AN 89127178 MEDLINE

L73 ANSWER 90 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI 2-CODON **MUTAGENESIS** OF GENE FOR **ALPHA-AMYLASE** IN **BACILLUS**-AMYLOLIQUEFACIENS
SO MOLECULAR BIOLOGY, (1988) Vol. 22, No. 5, pp. 1013-1019.
AU SMIRNOVA N A (Reprint); SOROKIN A V; LAPTEV D A; VEIKO V P; KOZLOV Y I
AN 89:321910 SCISEARCH

L73 ANSWER 91 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
TI **MUTATION** IN **ALPHA-AMYLASE** GENE OF **BACILLUS**-AMYLOLIQUEFACIENS LEADING TO REDUCTION IN TEMPERATURE OF PROTEIN INACTIVATION
SO MOLECULAR BIOLOGY, (1988) Vol. 22, No. 5, pp. 1006-1012.
AU SMIRNOVA N A (Reprint); SOROKIN A V; IOMANTAS Y V; ABALAKINA E G; KOZLOV Y I; STEPANOV A I
AN 89:321909 SCISEARCH

L73 ANSWER 92 OF 166 MEDLINE on STN DUPLICATE 33
TI [Regulation of **alpha-amylase** biosynthesis in **Bacillus** diastaticus **mutants** with various levels of enzyme synthesis].
Reguliatsiia biosinteza alpha-amilazy u mutantov Bacillus diastaticus s raznym urovnem sinteza fermenta.
SO MIKROBIOLOGIIA, (1988 Sep-Oct) 57 (5) 734-9.
Journal code: 0376652. ISSN: 0026-3656.
AU Murygina V P
AN 89261412 MEDLINE

L73 ANSWER 93 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Application of microbial amylases in food industry; glucose production using Aspergillus niger glucoamylase; characterization of **Bacillus** subtilis **alpha-amylase** for use in food industry (conference paper)
SO Biotechnol.Food.Ind.; (1988) 211-23
AU Wang J Y; Zhang S Z
AN 1990-05355 BIOTECHDS

L73 ANSWER 94 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Cloning of a starch degrading gene of B. megaterium; Bacillus megaterium (conference abstract)
SO Abstr.Annu.Meet.Am.Soc.Microbiol.; (1988) 88 Meet., 167
AU Klug C; Pun P; Vary P S
AN 1988-09948 BIOTECHDS

L73 ANSWER 95 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Studies on the technology of production of .**alpha**.-**amylase** by a **Mutant** B.S. 796
SO Shengwu Gongcheng Xuebao (1988), 4(2), 119-26
CODEN: SGXUED; ISSN: 1000-3061
AU Xu, Xianzhang; Gu, Chuanxian; Quan, Wenhai; Xu, Weiwei; Zhao, Yunlin; Xu, Yan; Chen, Rongxing; Wang, Jianwei
AN 1988:508870 HCAPLUS
DN 109:108870

L73 ANSWER 96 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Formation of some extracellular enzymes during the exponential growth of Bacillus subtilis;
neutral and alkaline protease and alpha-amylase
SO Folia Microbiol.; (1988) 33, 2, 88-95
CODEN: FOMIAZ
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AN 1988-09112 BIOTECHDS

L73 ANSWER 97 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
TI Processing and export of **Bacillus** stearothermophilus **alpha-amylase** carrying signal sequence **mutations**;
gene expression in Escherichia coli (conference paper)
SO ICSU Short Rep.; (1988) Vol.8, Miami Bio/Technol. Winter Symp., 38
AU Suominen I; Meyer P; Kopio A; Tiihonen R; Glumoff T; Karp M
AN 1989-11640 BIOTECHDS

L73 ANSWER 98 OF 166 MEDLINE on STN DUPLICATE 34
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Journal code: 2985088R. ISSN: 0022-2836.
AU Nicholson W L; Park Y K; Henkin T M; Won M; Weickert M J; Gaskell J A; Chambliss G H
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L73 ANSWER 99 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 35
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L73 ANSWER 100 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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L73 ANSWER 101 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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L73 ANSWER 103 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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L73 ANSWER 104 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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L73 ANSWER 106 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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L73 ANSWER 110 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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L73 ANSWER 115 OF 166 LIFESCI COPYRIGHT 2003 CSA on STNDUPLICATE 40
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L73 ANSWER 116 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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L73 ANSWER 117 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 119 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: BIOHAO
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L73 ANSWER 123 OF 166 MEDLINE on STN DUPLICATE 43
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L73 ANSWER 125 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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L73 ANSWER 126 OF 166 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
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L73 ANSWER 127 OF 166 MEDLINE on STN DUPLICATE 44
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L73 ANSWER 128 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 129 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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DN 103:193047

L73 ANSWER 130 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: EPXXDW
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DN 100:3412

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	EP 92235	A3	19840912		
	R: BE, CH, DE, FR, IT, LI, NL, SE				
	US 4465773	A	19840814	US 1982-370433	19820421
	AU 8313221	A1	19831027	AU 1983-13221	19830407
	AU 555540	B2	19861002		
	ZA 8302452	A	19831228	ZA 1983-2452	19830407
	IL 68378	A1	19860228	IL 1983-68378	19830414

	DK 8301733	A	19831022	DK 1983-1733	19830420
	GB 2118968	A1	19831109	GB 1983-10737	19830420
	GB 2118968	B2	19850717		
	JP 58190391	A2	19831107	JP 1983-69280	19830421

L73 ANSWER 131 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: EPXXDW
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	R: AT, BE, CH, DE, FR, GB, IT, LI, NL, SE				
	IL 66758	A1	19860228	IL 1982-66758	19820909
	DK 8204062	A	19830312	DK 1982-4062	19820910
	NO 8203080	A	19830314	NO 1982-3080	19820910
	ES 515620	A1	19840401	ES 1982-515620	19820910
	JP 58067186	A2	19830421	JP 1982-158725	19820911
	JP 62053150	B4	19871109		
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L73 ANSWER 134 OF 166 LIFESCI COPYRIGHT 2003 CSA on STN
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L73 ANSWER 135 OF 166 MEDLINE on STN DUPLICATE 46
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L73 ANSWER 136 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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L73 ANSWER 137 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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L73 ANSWER 138 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 139 OF 166 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
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PI RO 72541 A 19810820 (198239)*

L73 ANSWER 140 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 141 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 142 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: JKXXAF
AN 1980:468684 HCAPLUS
DN 93:68684

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 55034047	A2	19800310	JP 1978-106614	19780831
JP 62043677	B4	19870916		

L73 ANSWER 143 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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L73 ANSWER 144 OF 166 MEDLINE on STN DUPLICATE 48
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Biosintez alpha-amilazy variantami Bacillus subtilis.
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Journal code: 0376652. ISSN: 0026-3656.
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L73 ANSWER 145 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STNDUPLICATE 49
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L73 ANSWER 146 OF 166 MEDLINE on STN DUPLICATE 50
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L73 ANSWER 147 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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AN 79:443675 SCISEARCH

L73 ANSWER 148 OF 166 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
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L73 ANSWER 149 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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L73 ANSWER 150 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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DN 90:51250

L73 ANSWER 151 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Metody Poluch. Vysokoochishchennykh Fermentov (1978), 125-8. Editor(s): Glemzha, A. A. Publisher: Vses. Nauchno-Issled. Inst. Prikl. Enzimol., Vilnius, USSR.
CODEN: 43SAAT
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L73 ANSWER 152 OF 166 MEDLINE on STN
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AN 77254366 MEDLINE

L73 ANSWER 153 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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CODEN: ICHPCG; ISSN: 0379-4172
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DN 86:87645

L73 ANSWER 154 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Tutkimus ja Tekniikka (1976), (4), 56-7
CODEN: TTTKAR; ISSN: 0355-2578
AU Markkanen, Pertti
AN 1978:458323 HCAPLUS
DN 89:58323

L73 ANSWER 155 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
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SO Proizvodstvo i Primenenie Mikrobnykh Fermentnykh Preparatov (1976), 3, 12-23
CODEN: PPMPD6; ISSN: 0206-3425
AU Baranauskaite, A.; Bagdonaite, S.; Vasilauskas, J.; Galvydis, J.; Gorinchuk, G. F.
AN 1978:420053 HCAPLUS
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L73 ANSWER 156 OF 166 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
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AU Ayusawa D.; Yoneda Y.; Yamane K.; Maruo B.
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TI USE OF **MUTAGENIC** AGENTS IN IMPROVEMENT OF **ALPHA-AMYLASE** PRODUCTION BY **BACILLUS**-SUBTILIS
SO JOURNAL OF APPLIED CHEMISTRY AND BIOTECHNOLOGY, (1975) Vol. 25, No. 1, pp. 73-79.
AU BAILEY M J (Reprint); MARKKANEN P H
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L73 ANSWER 158 OF 166 MEDLINE on STN DUPLICATE 52
TI Mutation of **Bacillus** subtilis causing hyperproduction of **alpha-amylase** and protease, and its synergistic effect.
SO JOURNAL OF BACTERIOLOGY, (1975 Oct) 124 (1) 48-54.
Journal code: 2985120R. ISSN: 0021-9193.
AU Yoneda Y; Maruo B
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L73 ANSWER 159 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Properties of thermosensitive extracellular .**alpha**.-**amylases** of **Bacillus** subtilis
SO Journal of Bacteriology (1974), 120(2), 792-8
CODEN: JOBAAY; ISSN: 0021-9193
AU Yamane, Kunio; Maruo, Bunji
AN 1975:40031 HCAPLUS
DN 82:40031

L73 ANSWER 160 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Genetic and biochemical studies on cell-bound .**alpha**.-**amylase** in **Bacillus** subtilis
SO Journal of Bacteriology (1974), 119(2), 425-30
CODEN: JOBAAY; ISSN: 0021-9193
AU Nagata, Yoshiho; Yamaguchi, Kazuo; Maruo, Bunji
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DN 81:116909

L73 ANSWER 161 OF 166 MEDLINE on STN DUPLICATE 53
TI Isolation of **mutants** defective in **alpha-amylase** from **Bacillus** subtilis: genetic analyses.
SO JOURNAL OF BACTERIOLOGY, (1974 Aug) 119 (2) 416-24.
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AU Yamaguchi K; Nagata Y; Maruo B
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L73 ANSWER 162 OF 166 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
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SO JOURNAL OF BACTERIOLOGY, (1974) Vol. 119, No. 2, pp. 416-424.
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AN 74:318429 SCISEARCH

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TI Genetic control of the rate of .**alpha**. **amylase** synthesis in **Bacillus** subtilis.
SO Journal of Bacteriology, (1974) 119/2 (410-415).
CODEN: JOBAAY
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L73 ANSWER 164 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Membrane mutation related to the production of extracellular .**alpha**.-**amylase** and protease in **Bacillus** subtilis
SO Biochemical and Biophysical Research Communications (1973), 50(3), 765-70

CODEN: BBRCA9; ISSN: 0006-291X
AU Yoneda, Y.; Yamane, K.; Maruo, B.
AN 1973:94718 HCAPLUS
DN 78:94718

L73 ANSWER 165 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Regulation of .**alpha**.-**amylase** production in a **Bacillus** subtilis Marburg strain. I. Isolation of **mutants** which produce high levels of .**alpha**.-**amylase** and analysis of their enzymes
SO Hakko Kogaku Zasshi (1972), 50(12), 801-9
CODEN: HKZAA2; ISSN: 0367-5963
AU Sekiguchi, Junichi; Okada, Hirosuke
AN 1973:70185 HCAPLUS
DN 78:70185

L73 ANSWER 166 OF 166 HCAPLUS COPYRIGHT 2003 ACS on STN
TI The physical and genetic characterization of a transformable enzyme: **Bacillus** subtilis .**alpha**.-**amylase**
SO Biochimica et Biophysica Acta (1964), 89(2), 277-90
CODEN: BBACAQ; ISSN: 0006-3002
AU Green, MacDonald D.; Colarusso, Leonard J.
AN 1964:463499 HCAPLUS
DN 61:63499
OREF 61:11055c-d

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.84	265.49

STN INTERNATIONAL LOGOFF AT 14:38:13 ON 21 JUL 2003

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	372	(alpha adj amylase$1) same bacillus same (mutant$1 or variant$1)	USPAT; US-PGPUB	2003/07/21 12:18
2	L2	1028	(mutant$1 or variant$1) near5 (stability or thermostabilty or calcium adj depend$8)	USPAT; US-PGPUB	2003/07/21 12:19
3	L3	76	1 and 2	USPAT; US-PGPUB	2003/07/21 12:19

PGPUB-DOCUMENT-NUMBER: 20030129718

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129718 A1

TITLE: Amylase variants

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Andersen, Carsten	Vaerlose		DK	
Borchert, Torben Vedel	Birkerod		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	

APPL-NO: 09/ 925576

DATE FILED: August 9, 2001

RELATED-US-APPL-DATA:

child 09925576 A1 20010809

parent continuation-of PCT/DK01/00144 20010304 US UNKNOWN

non-provisional-of-provisional 60189857 20000315 US

non-provisional-of-provisional 60271382 20010226 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00376	2000DK-PA 2000 00376	March 8, 2000
DK	PA 2001 00303	2001DK-PA 2001 00303	February 23, 2001

US-CL-CURRENT: 435/183, 510/392

ABSTRACT:

The present invention relates to variants (mutants) of polypeptides, in particular Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency, specific activity, and solubility, in particular under production conditions.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/DK01/00144 filed Mar. 7, 2001 (the international application was published under PCT Article 21(2) in

English) and claims, under 35 U.S.C. 119, priority or the benefit of Danish application nos. PA 2000 00376 and PA 2001 00303 filed Mar. 8, 2000 and Feb. 23, 2001, respectively, and U.S. provisional application Nos. 60/189,857, and 60/271,382 filed Mar. 15, 2000 and Feb. 26, 2001, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (293):

[0330] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **alpha-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **alpha-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **alpha-amylase,** A. niger acid stable **alpha-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (374):

[0411] The assay can be used to screening of Termamyl-like .alpha.-amylase **variants having an improved stability** at high pH compared to the parent enzyme and Termamyl-like .alpha.-amylase **variants having an improved stability** at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting.

Detail Description Paragraph - DETX (409):

[0446] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed

Claims Text - CLTX (11):

11. The **variant** of any of claims 1-10, wherein the parent Termamyl-like **alpha-amylase** is derived from a strain of B. licheniformis, B. amyloliquefaciens, B. stearothermophilus, **Bacillus** sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, or DSMZ no. 12649, KSM AP1378, or KSM K36 or KSM K38.

PGPUB-DOCUMENT-NUMBER: 20030087794

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030087794 A1

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Wyoming	OH	US	
Meyer, Axel	Cincinnati	OH	US	

APPL-NO: 10/ 284665

DATE FILED: October 31, 2002

RELATED-US-APPL-DATA:

child 10284665 A1 20021031

parent continuation-of 09789884 20010221 US PENDING

non-provisional-of-provisional 60184268 20000223 US

US-CL-CURRENT: 510/499

ABSTRACT:

The present invention relates to liquid laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising:
a) from about 0.01 to about 20% by weight, of a zwitterionic polymer which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise an anionic moiety is less than or equal to the number of quaternized backbone amino units;
b) from about 0.1% to about 7% by weight, of a polyamine dispersant;
c) from about 0.01 % to about 80% by weight, of a surfactant system comprising one or more surfactants selected from the group consisting of nonionic, anionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof; and
d) the balance carriers and adjunct ingredients.

---------- KWIC ---------

Detail Description Paragraph - DETX (77):

[0167] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detail Description Paragraph - DETX (79):

[0169] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detail Description Paragraph - DETX (104):

[0194] Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are

preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, March 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M 197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

PGPUB-DOCUMENT-NUMBER: 20030064908

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030064908 A1

TITLE: Amylase variants

PUBLICATION-DATE: April 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bisgard-Frantzen, Henrik	Lyngby		DK	
Svendsen, Allan	Birkeroed		DK	
Borchert, Torben Vedel	Copenhagen N		DK	

APPL-NO: 10/ 025648

DATE FILED: December 19, 2001

RELATED-US-APPL-DATA:

child 10025648 A1 20011219

parent division-of 09902188 20010710 US PENDING

child 09902188 20010710 US

parent continuation-of 09354191 19990715 US GRANTED

parent-patent 6297038 US

child 09354191 19990715 US

parent continuation-of 08600656 19960213 US GRANTED

parent-patent 6093562 US

child 08600656 19960213 US

parent continuation-of PCT/DK96/00056 19960205 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0126/95	1995DK-0126/95	February 3, 1995
DK	0336/95	1995DK-0336/95	March 29, 1995
DK	1097/95	1995DK-1097/95	September 29, 1995
DK	1121/95	1995DK-1121/95	October 6, 1995

US-CL-CURRENT: 510/392, 435/183 , 510/530

ABSTRACT:

The present invention relates to variants of a parent .alpha.-amylase, which parent .alpha.-amylase (i) has an amino acid sequence selected from the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 7, respectively; or (ii) displays at least 80% homology with one or more of these amino acid sequences; and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of these amino acid sequences; and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an a-amylase having one of these amino acid sequences; in which variant:
(a) at least one amino acid residue of the parent .alpha.-amylase has been deleted; and/or
(b) at least one amino acid residue of the parent .alpha.-amylase has been replaced by a different amino acid residue; and/or
(c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency;
with the proviso that the amino acid sequence of the variant is not identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. patent application Ser. No. 09/902,188, filed on Jul. 10, 2001, which is a continuation of U.S. patent application Ser. No. 09/354,191, now U.S. Pat. No. 6,297,038, filed on Jul. 15,1999, which is a continuation of U.S. patent application Ser. No.08/600,656, now U.S. Pat. No. 6,093,562, filed on Feb. 13, 1996, which is a continuation of application serial no. PCT/DK96/00056, filed on Feb. 5,1996, which claims priority under 35 U.S.C. 119 of Danish application serial nos. 0126/95, filed on Feb. 3, 1995, 0336/95, filed on Mar. 29, 1995, 1097/95, filed on Sep. 29, 1995, and 1121/95, filed on Oct. 6, 1995, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):

[0008] WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Summary of Invention Paragraph - BSTX (13):

[0012] EP 525 610 relates to **mutant enzymes having improved stability** towards ionic tensides (surfactants). The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations are not specified.

Summary of Invention Paragraph - BSTX (14):

[0013] WO 94/02597 discloses .alpha.-amylase **mutants which exhibit improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Summary of Invention Paragraph - BSTX (17):

[0016] An object of the present invention is to provide .alpha.-amylase variants which--relative to their parent .alpha.-amylase--possess improved properties of importance, inter alia, in relation to the washing and/or dishwashing performance of the **variants in question, e.g. increased thermal stability,** increased stability towards oxidation, reduced dependency on Ca.sup.2+ ion and/or improved stability or activity in the pH region of relevance in, e.g., laundry washing or dishwashing. Such variant .alpha.-amylases have the advantage, among others, that they may be employed in a lower dosage than their parent .alpha.-amylase. Furthermore, the .alpha.-amylase variants may be able to remove starchy stains which cannot, or can only with difficulty, be removed by .alpha.-amylase detergent enzymes known today.

Summary of Invention Paragraph - BSTX (92):

[0089] From the results obtained by the present inventors it appears that changes in a particular property, e.g. thermal **stability or oxidation stability, exhibited by a variant** relative to the parent .alpha.-amylase in question can to a considerable extent be correlated with the type of, and positioning of, mutation(s) (amino acid substitutions, deletions or insertions) in the variant. It is to be understood, however, that the observation that a particular mutation or pattern of mutations leads to changes in a given property in no way excludes the possibility that the mutation(s) in question can also influence other properties.

Summary of Invention Paragraph - BSTX (93):

[0090] Oxidation stability: With respect to increasing the oxidation **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable that at least one, and preferably multiple, oxidizable amino acid residue(s) of the parent has/have been deleted or replaced (i.e. substituted by) a different amino acid residue which is less susceptible to oxidation than the original oxidizable amino acid residue.

Summary of Invention Paragraph - BSTX (94):
[0091] Particularly relevant oxidizable amino acid residues in this connection are cysteine, methionine, tryptophan and tyrosine. Thus, for example, in the case of parent .alpha.-amylases containing cysteine it is anticipated that deletion of cysteine residues, or substitution thereof by less oxidizable amino acid residues, will be of importance in obtaining **variants with improved oxidation stability** relative to the parent .alpha.-amylase.

Summary of Invention Paragraph - BSTX (95):
[0092] In the case of the above-mentioned parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No.1, SEQ ID No.2 and SEQ ID No. 7, respectively, all of which contain no cysteine residues but have a significant methionine content, the deletion or substitution of methionine residues is particularly relevant with respect to achieving improved oxidation **stability of the resulting variants**. Thus, deletion or substitution [e.g. by threonine (T), or by one of the other amino acids listed above] of one or more of the methionine residues in positions M9, M10, M105, M202, M208, M261, M309, M382, M430 and M440 of the amino acid sequences shown in SEQ ID No.1, SEQ ID No.2 and SEQ ID No.7, and/or in position M323 of the amino acid sequence shown in SEQ ID No. 2 (or deletion or substitution of methionine residues in equivalent positions in the sequence of another .alpha.-amylase meeting one of the other criteria for a parent .alpha.-amylase mentioned above) appear to be particularly effective with respect to increasing the oxidation stability.

Summary of Invention Paragraph - BSTX (104):
[0101] Thermal stability: With respect to increasing the thermal **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ ID No.1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in SEQ ID No.2, SEQ ID No. 3 and SEQ ID No. 7, respectively, are: F180, R181, G182, D183, G184 and K185 (SEQ ID No.2); F178, R179, G180,1181, G182 and K183 (SEQ ID No. 3); and F180, R181, G182, H183, G184 and K185 (SEQ ID No. 7).

Summary of Invention Paragraph - BSTX (112):
[0109] Examples of specific mutations which appear to be of importance in connection with the thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequence shown in SEQ ID No.1 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): K269R; P260E; R124P; M105F,I,L,V; M208F,W,Y; L217I; V206I,L,F.

Summary of Invention Paragraph - BSTX (116):
[0113] Still further examples of mutations which appear to be of importance, interalia, in achieving improved thermal **stability of an .alpha.-amylase**

variant relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): A354C+V479C; L351C+M430C; N457D,E+K385R; L355D,E+M430R,K; L355D,E+1411R,K; and N457D,E.

Summary of Invention Paragraph - BSTX (209):

[0206] .alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.quadrature. or 65.quadrature.C for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No.1 can barely be detected. Stabilized variants show, under the same conditions, increased color intensitydue to increased liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (219):

[0216] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucormiehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (55):

[0354] Determination of oxidation **stability of M202 substitution variants** of the parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No.1 and SEQ ID No. 2 A: Oxidation **stability of variants** of the sequence in SEQ ID No.1

Detail Description Paragraph - DETX (60):

[0359] B: Oxidation **stability of variants** of the sequence in SEQ ID No.2

Detail Description Paragraph - DETX (64):

[0362] Determination of thermal **stability f variants** of the parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No.1 and SEQ ID No.2 A: Thermal **stability of pairwise deletion variants** of the sequence in SEQ ID No.1

Detail Description Paragraph - DETX (72):

[0370] It is apparent that all of the pairwise deletion variants tested exhibit significantly improved thermal stability relative to the parent .alpha.-amylase (SEQ ID No. 1), and that the thermal **stability of Variant** 5, which in addition to the pairwise deletion mutation of Variant 4 comprises the substitution R124P, is markedly higher than that of the other variants. Since calorimetric results for the substitution variant R124P (comprising only the substitution R124P) reveal an approximately 7.quadrature.C thermostabilization thereof relative to the parent .alpha.-amylase, it appears that the thermostabilizing effects of the mutation R124P and the pairwise deletion, respectively, reinforce each other.

Detail Description Paragraph - DETX (73):

[0371] B: Thermal **stability of pairwise deletion variants** of the sequence in SEQ ID No. 2

Detail Description Paragraph - DETX (79):

[0377] C: Thermal **stability of a multi-combination variant** of the sequence in SEQ ID No.1

PGPUB-DOCUMENT-NUMBER: 20030059902

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030059902 A1

TITLE: Amylolytic enzyme variants

PUBLICATION-DATE: March 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cherry, Joel Robert	Davis	CA	US	
Svendsen, Allan	Birkerod		DK	
Andersen, Carsten Denmark	Vaerloese		DK	
Beier, Lars	Lyngby		DK	
Frandsen, Torben Peter	Frederiksberg C		DK	
Schafer, Thomas	Farum		DK	

APPL-NO: 10/ 234266

DATE FILED: September 4, 2002

RELATED-US-APPL-DATA:

child 10234266 A1 20020904

parent division-of 09645707 20000824 US PENDING

child 09645707 20000824 US

parent continuation-of PCT/DK99/00087 19990226 US UNKNOWN

non-provisional-of-provisional 60077509 19980311 US

non-provisional-of-provisional 60077795 19980312 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1998 00269	1998DK-1998 00269	February 27, 1998
DK	1998 00273	1998DK-1998 00273	February 27, 1998

US-CL-CURRENT: 435/101, 435/193 , 435/202 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The inventors have discovered some striking, and not previously predicted structural similarities and differences between the structure of Novamyl and the reported structures of CGTases, and based on this they have constructed variants of maltogenic alpha-amylase having CGTase activity and variants of

CGTase having maltogenic alpha-amylase activity. Further, on the basis of sequence homology between Novamyl.RTM. and CGTases, the inventors have constructed hybrid enzymes with one or more improvements to specific properties of the parent enzymes, using recombinant DNA methodology.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. Ser. No. 09/645,707, filed on Aug. 24, 2000 (now allowed), which is a continuation of PCT/DK/99/00087, filed on Feb. 26, 1999, and claims priority under 35 U.S.C. 119 of Danish application nos. PA 1998 00269 and PA 1998 00273, both filed on Feb. 27, 1998, and U.S. provisional application Nos. 60/077,509 and 60/077,795, filed on Mar. 11, 1998 and Mar. 12, 1998, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (95):

[0109] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence.. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a maltogenic **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (111):

[0124] To screen for **variants with increased stability,** the filter with bound maltogenic alpha-amylase variants can be pretreated prior to the detection step described above to inactivate **variants that do not have improved stability** relative to the parent CGTase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection

media.

Detail Description Paragraph - DETX (112):

[0125] To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent CGTase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Red labeled amylopectin and incubated until activity is detectable. Similarly, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent CGTase is inactivated, thereby allowing detection of only those **variants with increased stability** at the pH in question. To screen for **variants with increased calcium-dependent** stability calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent CGTase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detail Description Paragraph - DETX (166):

[0163] In this example, the unique active site loop was used to select hybrid enzymes with maltogenic **alpha-amylase** activity from a library of random recombinants. In this method, Novamyl and the cyclic maltodextrin glucosyl transferase (CGTase) from **Bacillus** circulans, were randomly recombined by the DNA shuffling method of Crameri A, et al., op.cit. Those resulting **mutants** containing the Novamyl loop were selected using PCR as described above in Example 2.

PGPUB-DOCUMENT-NUMBER: 20030044954

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030044954 A1

TITLE: Alpha-amylase variants

PUBLICATION-DATE: March 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Andersen, Carsten	Vaerlose		DK	
Jorgensen, Christel Thea	Kobenhavn O		DK	
Bisgard-Frantzen, Henrik	Bagsvaerd		DK	
Svendsen, Allan	Birkerod		DK	
Kjaerulff, Soren	Vanlose		DK	

APPL-NO: 10/ 146327

DATE FILED: May 15, 2002

RELATED-US-APPL-DATA:

child 10146327 A1 20020515

parent division-of 09537168 20000329 US GRANTED

parent-patent 6410295 US

non-provisional-of-provisional 60127427 19990401 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	1999 00437	1999DK-1999 00437	March 30, 1999

US-CL-CURRENT: 435/202, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like alpha-amylase, which variant exhibits altered properties, in particular reduced capability of cleaving a substrate close to the branching point, and improved substrate specificity and/or improved specific activity relative to the parent alpha-amylase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a division of U.S. application Ser. No. 09/537,168 filed Mar. 29, 2000, and claims, under 35 U.S.C. 119, priority of Danish application no. PA 1999 00437 filed Mar. 30, 1999, and claims the benefit of

U.S. provisional No. 60/127,427 filed on Apr. 1, 1999 the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (6):

[0005] Among recent disclosure relating to **alpha-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **alpha-amylase,** reffered to as BA2, which consists of the 300 N-terminal amino acid residues of the B. amytoliquefaciens **alpha-amylase** comprising the amino acid sequence shown in SEQ ID NO: 6 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis **alpha-amylase** comprising the amino acid sequence shown in SEQ ID NO: 4 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus alpha-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **alpha-amylases**", and which include, inter alia, the B. licheniformis, B. amytoliquefaciens and B. stearothermophilus **alpha-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **alpha-amylase, variants** of the parent Termamyl-like **alpha-amylase** which exhibit altered properties relative to the parent.

Summary of Invention Paragraph - BSTX (237):

[0234] (c) screening for host cells expressing an alpha-amylase **variant which has an altered property (i.e., thermal stability)** relative to the parent alpha-amylase.

Summary of Invention Paragraph - BSTX (247):

[0244] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **alpha-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **alpha-amylase** (amyQ), the promoters of the **Bacillus** subfilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **alpha-amylase,** A. niger acid stable **alpha-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

PGPUB-DOCUMENT-NUMBER: 20030022346

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030022346 A1

TITLE: Novel amylolytic enzymes derived from the B.licheniformis alpha-amylase, having improved characteristics

PUBLICATION-DATE: January 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Van Der Laan, Jan M.	Breda		NL	
Aehle, Wolfgang	Delft		NL	

APPL-NO: 10/ 199922

DATE FILED: July 18, 2002

RELATED-US-APPL-DATA:

child 10199922 A1 20020718

parent continuation-of 08981184 19971211 US ABANDONED

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
EP	94201740.1	1994EP-94201740.1	June 17, 1994

US-CL-CURRENT: 435/202, 435/252.31 , 435/320.1 , 435/69.1 , 510/305 , 536/23.2

ABSTRACT:

The present invention relates to novel amylolytic enzymes having improved characteristics for the use in starch degradation, in textile or paper desizing and in household detergent compositions. The disclosed .alpha.-amylases show surprisingly improved properties with respect to the activity level and the combination of thermostability and a higher activity level. These improved properties make them more suitable for the use under more acidic or more alkaline conditions. The improved properties allow also the reduction of the Calcium concentration under application conditions without a loss of performance of the enzyme.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):

[0019] In a further embodiment of the invention, the **mutants of the invention are combined with mutations which improve the oxidation stability** of the amylolytic enzyme. Such mutant enzymes may comprise mutations known in the art to improve the oxidation stability of amylolytic enzymes, such as e.g. mutations which replace the methionine at position 197 (see e.g. PCT/DK93/00230).

Detail Description Paragraph - DETX (6):

[0035] The B.licheniformis **.alpha.-amylase** gene used throughout this study was obtained from plasmid pMcTLia6 (WO91/00353) as an EcoRI-HinDIII restriction fragment still including the inducible Tac promoter. This fragment was inserted in EcoRI-HinDIII digested pBHA1 to yield plasmid pBHATLAT (FIG. 2). This plasmid is used for the expression of **.alpha.-amylase** in E.coli through induction of the Tac promoter by 0.2 mM IPTG. Expression of **mutant .alpha.-amylase** was obtained by replacing the wild type **.alpha.-amylase** gene fragment by the corresponding **mutant** gene fragment. For expression in **Bacillus**, plasmid pBHATLAT was digested with BamHI and subsequent relegation thus placing the **.alpha.-amylase** gene under the control of the constitutive Hpall promoter. Wild type and **mutant .alpha.-amylase** enzyme was isolated from the **Bacillus** culture supernatant.

PGPUB-DOCUMENT-NUMBER: 20020192792

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020192792 A1

TITLE: Laccase mutants

PUBLICATION-DATE: December 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schneider, Palle	Ballerup		DK	
Danielsen, Steffen	Copenhagen		DK	
Svendsen, Allan	Hoersholm		DK	

APPL-NO: 09/ 869877

DATE FILED: July 6, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2001 00327	2001DK-PA 2001 00327	February 28, 2001
DK	PA 2000 00707	2000DK-PA 2000 00707	April 28, 2000

PCT-DATA:
APPL-NO: PCT/DK01/00292
DATE-FILED: Apr 30, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/200, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

By analyzing the three-dimensional structure of the Coprinus laccase structural parts or specific amino acid residues can be identified, which from structural or functional considerations appear to be important for the oxidative stability of a laccase. When comparing the three-dimensional structure of the Coprinus laccase structure with known amino acid sequences of various laccases, it has been found that several similarities exist between the sequences.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a 35 U.S.C. 371 national application of PCT/DK01/00292 filed Apr. 30, 2001 and claims, under 35 U.S.C. 119, priority or the benefit of Danish application nos. PA 2000 00707 and PA 2001 00327 filed Apr. 28, 2000 and Feb. 28, 2001, respectively, and U.S. application

Nos. 60/203,345 and 60/277,817 filed May 10, 2000 and Mar. 21, 2001, respectively, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):
[0002] The invention relates to laccase **mutants with improved stability** properties.

Summary of Invention Paragraph - BSTX (6):
[0005] Accordingly, it is the purpose of the present invention to create laccase **variants with improved oxidative stability** by using the information of a three-dimensional structure of a Coprinus cinereus laccase.

Detail Description Paragraph - DETX (36):
[0043] The laccase mutants of the present invention may be designed by constructing a variant of a parent Coprinus laccase, which **variant has laccase activity and improved stability** as compared to the parent laccase, which method comprises:

Detail Description Paragraph - DETX (39):
[0046] iii) testing the resulting Coprinus laccase **variant with respect to stability**.

Detail Description Paragraph - DETX (40):
[0047] The laccase mutants of the invention may also be designed by constructing a variant of a parent Coprinus-like laccase, which **variant has laccase activity and improved stability** as compared to the parent laccase, which method comprises:

Detail Description Paragraph - DETX (43):
[0050] iii) modifying the part of the Coprinus-like laccase identified in ii) whereby a Coprinus-like laccase **variant is obtained, which has an improved stability** as compared to the parent Coprinus-like laccase, and optionally,

Detail Description Paragraph - DETX (44):
[0051] iv) testing the resulting Coprinus-like laccase **variant with respect to stability**.

Detail Description Paragraph - DETX (47):
[0054] The laccase **variants of the invention have improved oxidative stability** compared to the un-modified parent laccases. Improved oxidative **stability means that the laccase variants** of the invention have improved

tolerance towards oxidative chemical compounds, such as radicals formed from laccase mediated oxidation of radical precursor compounds. The radical precursor compounds may preferably be mediators or "enhancing agents", such as those described in EP 705327 (compounds containing N--OH, N--O and NR--OH groups), WO9501426 (compounds containing two aromatic rings etc.), WO 96/10079 (methylsyringate type of compounds) and/or WO 99/57360 (N-hydroxyacetanilide type of compounds).

Detail Description Paragraph - DETX (55):
[0062] **Variants with Altered Oxidative Stability**

Detail Description Paragraph - DETX (109):
[0116] Suitable amylases (.alpha. and/or .beta.) include those of bacterial or fungal origin. Chemically modified or protein engineered **mutants** are included. Amylases include, for example, **.alpha.-amylases** obtained from **Bacillus**, e.g. a special strain of B. licheniformis, described in more detail in GB 1,296,839.

Detail Description Paragraph - DETX (169):
[0176] The stability against oxidation by radicals (oxidative **stability) of Coprinus variants** or Coprinus-like variants may be measured as described in the following.

PGPUB-DOCUMENT-NUMBER: 20020187528

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020187528 A1

TITLE: Fermentation with a phytase

PUBLICATION-DATE: December 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Veit, Chris	Wake Forest	NC	US	
Felby, Claus	Herlev	NC	DK	
Peckous, Larry W.	Raleigh		US	
Olsen, Hans Sejr	Holte		DK	

APPL-NO: 10/ 164029

DATE FILED: June 6, 2002

RELATED-US-APPL-DATA:

child 10164029 A1 20020606

parent continuation-of 09788906 20010220 US PENDING

non-provisional-of-provisional 60185716 20000223 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00281	2000DK-PA 2000 00281	February 23, 2000

US-CL-CURRENT: 435/105, 435/155 , 435/254.21

ABSTRACT:

The present invention relates to an improved fermenation process wherein phytic acid-containing material is fermented in the presence of a phytase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/788,906, filed on Feb. 20, 2001, and claims the benefit of U.S. Provisional application No. 60/185,716, filed Feb. 23, 2000, and priority under 35 U.S.C. 119 of Danish application PA 2000 00281, filed Feb. 23, 2000, the contents of which are fully incorporated herein by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (66):

[0072] The liquefaction step may be performed in the presence of an **alpha-amylase** derived from a microorganism or a plant. Preferred **alpha-amylases** are of fungal or bacterial origin. **Bacillus alpha-amylases** (often referred to as "Termamyl-like **alpha-amylases"), variant** and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like **alpha-amylases** include **alpha-amylase** derived from a strain of B. licheniformis (commercially available as Termamyl.TM.), B. amyloliquefaciens, and B. stearothermophilus **alpha-amylase** (BSG). Other Termamyl-like **alpha-amylases** include **alpha-amylase** derived from a strain of the **Bacillus** sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the **alpha-amylase** described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention, a Termamyl-like **alpha-amylase is an alpha-amylase** as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated **variants** and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Contemplated **alpha-amylase** derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger **alpha-amylases**. Commercial **alpha-amylase** products and products containing **alpha-amylases** include TERMAMYL.TM. SC, FUNGAMYL.TM., LIQUOZYME.TM. and SAN.TM. SUPER.

Detail Description Paragraph - DETX (71):

[0077] Other suitable Aspergillus glucoamylase **variants include variants to enhance the thermal stability**: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct. 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct. 12-17, 1997, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability. Other glucoamylases include Talaromyces glucoamylases, preferably derived from Talaromyces emersonii (WO 99/28448), Talaromyces leycettanus (U.S. Pat. No. Re. 32,153), Talaromyces duponti, Talaromyces thermophilus (U.S. Pat. No. 4,587,215). Bacterial glucoamylases preferably include glucoamylases from the genus Clostridium, more preferably, C. thermoamylolyticum (EP 135,138), and C. thermohydrosulfuricum (WO 86/01831).

PGPUB-DOCUMENT-NUMBER: 20020182672

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020182672 A1

TITLE: Enhanced secretion of a polypeptide by a microorganism

PUBLICATION-DATE: December 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kolkman, Marc	Oegstgeest		NL	

APPL-NO: 09/ 975132

DATE FILED: October 9, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60239531 20001010 US

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/252.31

ABSTRACT:

Described herein are methods for the enhanced production of secreted proteins. The secretion of a protein of interest having a substantially non-polar carboxy tail is enhanced by the placement of charged amino acid residues at the carboxy terminus either by adding to the native peptide or by replacing, i.e., substituting, the terminal residues of the native peptide.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. .sctn.119(e), the present application claims benefit of and priority to U.S. Ser. No. 60/239,531, entitled "Enhanced Secretion of a Polypeptide by a Microorganism", filed Oct. 10, 2000, by Marc Kolkman.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (4):
[0014] FIG. 3. **Stability of hIL-3 variants** with different C-terminal tags.

Detail Description Paragraph - DETX (90):
[0107] Plasmid pLATIL3, a derivative of pGB/IL-322, contains an expression cassette for the production of human interleukin-3 (hIL-3) by Bacilli (Van Leen et al. 1991). In this construct, the B. licheniformis **.alpha.-amylase** (AmyL)

signal peptide is used to direct secretion of mature hIL-3. As a model for SsrA-mediated peptide tagging in B. subtilis, a **variant** of plasmid pLATIL3 was created in which a transcription terminator is inserted into the AmyL-hIL3 gene, just in front of its stop codon. Transformation of this plasmid (pLATIL3TERM) into B. subtilis will result in AmyL-hIL3 transcripts lacking in-frame stop codons. According to the tmRNA model for SsrA mediated tagging of proteins (Keiler et al. 1996), translation of these transcript will result in ribosome stalling, and subsequently recruitment of SsrA, peptide tagging, and finally degradation of the tagged hIL-3 molecules by specific proteases. To test this model in **Bacillus,** the extracellular proteins produced in cultures of B. subtilis 168 (pLATIL3TERM), 168 .DELTA.ssrA (pLATIL3TERM), and the control strain 168 (pLATIL3), were analyzed by Western blotting (FIG. 2). Human IL-3 accumulated in the medium of strain 168 .DELTA.ssrA (pLATIL3TERM), but could not be detected in the medium of B. subtilis 168 (pLATIL3TERM) containing functional SsrA. These data indicate that B. subtilis SsrA has a role in a process in which proteins translated from mRNAs lacking an in-frame stop codon are degraded. In contrast, in cells without SsrA the hIL-3 molecules are released from stalled ribosomes by an SsrA-independent mechanism (see below). These molecules do not receive a peptide-tag and, therefore are not rapidly degraded by B. subtilis.

Detail Description Paragraph - DETX (97):

[0112] **Stability of hIL-3 Variants** with Different C-Terminal Tags Produced by B. Subtilis.

PGPUB-DOCUMENT-NUMBER: 20020165111

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020165111 A1

TITLE: LAUNDRY DETERGENT COMPOSITIONS COMPRISING ZWITTERIONIC POLYAMINES AND XYLOGLUCANASE

PUBLICATION-DATE: November 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ghosh, Chanchal Kumar	West Chester	OH	US	

APPL-NO: 09/ 790044

DATE FILED: February 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60184367 20000223 US

US-CL-CURRENT: 510/320, 510/319 , 510/392 , 510/499

ABSTRACT:

The present invention solves the problem of soil and dirt becoming entrained in cellulosic material loosened and removed from fabric during washing wherein said dirt and soil is entrapped by the cellulosic material and re-deposited onto the fabric surface. The compositions of the present invention comprise:
a) from about 0.01% by weight, of a zwitterionic polyamine which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise said anionic moiety is less than or equal to the number of quaternized backbone amino units;
b) from about 0.00005% by weight, of a xyloglucanase enzyme;
c) from about 0.5% to about 50% by weight, of a surfactant system comprising:
i) from about 10% to about 99% by weight, of said surfactant system, of a nonionic surfactant;
ii) from about 1% to about 90% by weight, of said surfactant system, of an anionic surfactant;
iii) optionally, from 1% to about 50% by weight, of said surfactant system, of a detersive surfactant selected from the group consisting of cationic surfactants, zwitterionic surfactants, ampholytic surfactants, and mixtures thereof; and
d) the balance carriers and adjunct ingredients.

CROSS REFERENCE

[0001] This Application claims the benefit of U.S. Provisional Application No. 60/184,367, filed on Feb. 23, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (90):

[0188] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detail Description Paragraph - DETX (92):

[0190] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detail Description Paragraph - DETX (124):

[0221] Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be

obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

PGPUB-DOCUMENT-NUMBER: 20020155574

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020155574 A1

TITLE: Alpha-amylase mutants with altered properties

PUBLICATION-DATE: October 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Thisted, Thomas	Rungsted Kyst		DK	
Kjaerulff, Soren	Vanlose		DK	
Andersen, Carsten	Vaerloese		DK	
Fuglsang, Claus Crone	Niva		DK	

APPL-NO: 09/ 918543

DATE FILED: July 31, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60225140 20000814 US

non-provisional-of-provisional 60233986 20000920 US

non-provisional-of-provisional 60249104 20001116 US

non-provisional-of-provisional 60286869 20010426 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 01160	2000DK-PA 2000 01160	August 1, 2000
DK	PA 2000 01354	2000DK-PA 2000 01354	September 12, 2000
DK	PA 2000 01687	2000DK-PA 2000 01687	November 10, 2000
DK	PA 2001 00655	2001DK-PA 2001 00655	April 26, 2001

US-CL-CURRENT: 435/202, 435/203 , 435/320.1 , 435/325 , 435/69.1

ABSTRACT:

The present invention relates to variants (mutants) of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits altered stability, in particular at high temperatures and/or at low pH relative, and/or low Ca2+ to the parent alpha-amylase.

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Detail Description Paragraph - DETX (94):

[0125] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **alpha-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **alpha-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **alpha-amylase,** A. niger acid stable **alpha-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (137):

[0168] **Stability Assay of Unpurified Variants**:

Detail Description Paragraph - DETX (138):

[0169] **Bacillus** cultures expressing the **variants** to be analysed are grown for 21 hours at 37.degree. C. in 10 ml LB+chloramphenicol. 800 micro liter culture is mixed with 200 micro 1 citrate buffer, pH 4.5. A number of 70 micro 1 aliquots corresponding to the number of sample time points are made in PCR tubes and incubated at 70.degree. C. (for **variants** in the wt backbone) or 90.degree. C. (for **variants** in LE399) for various time points (typically 5, 10, 15, 20, 25 and 30 minutes) in a PCR machine. The 0 min sample is not incubated at high temperature. Activity in the sample is measured by transferring 20 micro 1 to 200 micro 1 of the **alpha-amylase** PNP-G7 substrate MPR3 ((Boehringer Mannheim Cat. no. 1660730) as described below under "Assays for **Alpha-Amylase** Activity". Results are plotted as percentage activity (relative to the 0 time point) versus time, or stated as percentage residual activity after incubation for a certain period of time.

Detail Description Paragraph - DETX (143):

[0174] **Stability Determination of Purified Variants**

Detail Description Paragraph - DETX (144):

[0175] All **stability trials of purified variants** are made using the same set up. The method is as follows: The enzyme is incubated under the relevant conditions (1-4). Samples are taken at various time points, e.g., after 0, 5, 10, 15 and 30 minutes and diluted 25 times (same dilution for all taken samples) in assay buffer (0.1 M 50 mM Britton buffer pH 7.3) and the activity is measured using the Phadebas assay (Pharmacia) under standard conditions pH 7.3, 37.degree. C.

Detail Description Paragraph - DETX (159):

[0188] Construction, by error-prone PCR mutagenesis, of **Bacillus** licheniformis **alpha-amylase variants having an improved stability** at low pH, high temperature and low calcium ion concentration compared to the parent enzyme.

Claims Text - CLTX (12):

12. The variant of any of claims 1-11, which **variant has altered stability,** in particular at high temperatures from 70-120.degree. C. and/or low pH in the range from pH 4-6.

PGPUB-DOCUMENT-NUMBER: 20020123123

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020123123 A1

TITLE: Cutinase variants

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Horsholm		DK	
Glad, Sanne O. Schroder	Ballerup		DK	
Fukuyama, Shiro	Chiba		JP	
Matsui, Tomoko	Chiba		JP	

APPL-NO: 09/ 873075

DATE FILED: June 1, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60211004 20000612 US

non-provisional-of-provisional 60244351 20001030 US

non-provisional-of-provisional 60253798 20001129 US

non-provisional-of-provisional 60265473 20010131 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00861	2000DK-PA 2000 00861	June 2, 2000
DK	PA 2000 01577	2000DK-PA 2000 01577	October 23, 2000
DK	PA 2000 01772	2000DK-PA 2000 01772	November 24, 2000
DK	PA 2001 00100	2001DK-PA 2001 00100	January 19, 2001

US-CL-CURRENT: 435/200, 435/252.3 , 435/254.2 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

Variants of fungal cutinases having improved thermostability comprise substitution of one or more specified amino acid residues and/or a specified N-terminal extension. The variants may optionally comprise additional alterations at other positions.

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Summary of Invention Paragraph - BSTX (82):

[0077] Examples of suitable promoters for directing the transcription of the DNA sequence encoding a cutinase **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, the TPI (triose phosphate isomerase) promoter from S. cerevisiae (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, Rhizomucormieheiaspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (24):

Stability of Variants in Presence of Detergent

Detail Description Paragraph - DETX (50):

[0225] Further, the **stability of the two variants** was determined in solutions of the two detergents. After 10 minutes at 25.degree. C., the variant of the invention showed 92-95% residual activity in the two detergents, whereas the reference variant showed 71-72% residual activity. The results showed that the variant of the invention provides a clearly improved washing performance in terms of washing performance, anti-redeposition effect and stability in detergent solution.

PGPUB-DOCUMENT-NUMBER: 20020098996

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020098996 A1

TITLE: Amylase variants

PUBLICATION-DATE: July 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bisgard-Frantzen, Henrik	Lyngby		DK	
Svendsen, Allan	Birkeroed		DK	
Borchert, Torben Vedel	Copenhagen N		DK	

APPL-NO: 09/ 902188

DATE FILED: July 10, 2001

RELATED-US-APPL-DATA:

child 09902188 A1 20010710

parent continuation-of 09354191 19990715 US GRANTED

parent-patent 6297038 US

child 09354191 19990715 US

parent continuation-of 08600656 19960213 US GRANTED

parent-patent 6093562 US

child 08600656 19960213 US

parent continuation-of PCT/DK96/00056 19960205 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	0126/95	1995DK-0126/95	February 3, 1995
DK	0336/95	1995DK-0336/95	March 29, 1995
DK	1097/95	1995DK-1097/95	September 29, 1995
DK	1121/95	1995DK-1121/95	October 6, 1995

US-CL-CURRENT: 510/392

ABSTRACT:

The present invention relates to variants of a parent .alpha.-amylase, which parent .alpha.-amylase (i) has an amino acid sequence selected from the amino

acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 7, respectively; or (ii) displays at least 80% homology with one or more of these amino acid sequences; and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of these amino acid sequences; and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an .alpha.-amylase having one of these amino acid sequences; in which variant: (a) at least one amino acid residue of the parent .alpha.-amylase has been deleted; and/or (b) at least one amino acid residue of the parent .alpha.-amylase has been replaced by a different amino acid residue; and/or (c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency; with the proviso that the amino acid sequence of the variant is not identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively.

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Summary of Invention Paragraph - BSTX (9):

[0007] WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Summary of Invention Paragraph - BSTX (13):

[0011] EP 525 610 relates to **mutant enzymes having improved stability** towards ionic tensides (surfactants). The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations are not specified.

Summary of Invention Paragraph - BSTX (14):

[0012] WO 94/02597 discloses .alpha.-amylase **mutants which exhibit improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Summary of Invention Paragraph - BSTX (17):

[0015] An object of the present invention is to provide .alpha.-amylase variants which--relative to their parent .alpha.-amylase--possess improved properties of importance, inter alia, in relation to the washing and/or dishwashing performance of the **variants in questi n, e.g. increased thermal stability,** increased stability towards oxidation, reduced dependency on Ca.sup.2+ ion and/or improved stability or activity in the pH region of relevance in, e.g., laundry washing or dishwashing. Such variant .alpha.-amylases have the advantage, among others, that they may be employed in a lower dosage than their parent .alpha.-amylase. Furthermore, the .alpha.-amylase variants may be able to remove starchy stains which cannot, or can only with difficulty, be removed by .alpha.-amylase detergent enzymes known today.

Summary of Invention Paragraph - BSTX (93):

[0089] From the results obtained by the present inventors it appears that changes in a particular property, e.g. thermal **stability or oxidation stability, exhibited by a variant** relative to the parent .alpha.-amylase in question can to a considerable extent be correlated with the type of, and positioning of, mutation(s) (amino acid substitutions, deletions or insertions) in the variant. It is to be understood, however, that the observation that a particular mutation or pattern of mutations leads to changes in a given property in no way excludes the possibility that the mutation(s) in question can also influence other properties.

Summary of Invention Paragraph - BSTX (94):

[0090] Oxidation stability: With respect to increasing the oxidation **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable that at least one, and preferably multiple, oxidizable amino acid residue(s) of the parent has/have been deleted or replaced (i.e. substituted by) a different amino acid residue which is less susceptible to oxidation than the original oxidizable amino acid residue.

Summary of Invention Paragraph - BSTX (95):

[0091] Particularly relevant oxidizable amino acid residues in this connection are cysteine, methionine, tryptophan and tyrosine. Thus, for example, in the case of parent .alpha.-amylases containing cysteine it is anticipated that deletion of cysteine residues, or substitution thereof by less oxidizable amino acid residues, will be of importance in obtaining **variants with improved oxidation stability** relative to the parent .alpha.-amylase.

Summary of Invention Paragraph - BSTX (96):

[0092] In the case of the above-mentioned parent .alpha.-amylases having the amino acid sequences shown in SEQ. ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, respectively, all of which contain no cysteine residues but have a significant methionine content, the deletion or substitution of methionine residues is particularly relevant with respect to achieving improved oxidation **stability of the resulting variants**. Thus, deletion or substitution [e.g. by threonine

(T), or by one of the other amino acids listed above] of one or more of the methionine residues in positions M9, M10, M105, M202, M208, M261, M309, M382, M430 and M440 of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, and/or in position M323 of the amino acid sequence shown in SEQ ID No. 2 (or deletion or substitution of methionine residues in equivalent positions in the sequence of another .alpha.-amylase meeting one of the other criteria for a parent .alpha.-amylase mentioned above) appear to be particularly effective with respect to increasing the oxidation stability.

Summary of Invention Paragraph - BSTX (105):

[0101] Thermal stability: With respect to increasing the thermal **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ ID No. 1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively, are: F180, R181, G182, D183, G184 and K185 (SEQ ID No. 2); F178, R179, G180, I181, G182 and K183 (SEQ ID No. 3); and F180, R181, G182, H183, G184 and K185 (SEQ ID No. 7).

Summary of Invention Paragraph - BSTX (113):

[0109] Examples of specific mutations which appear to be of importance in connection with the thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequence shown in SEQ ID No. 1 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): K269R; P260E; R124P; M105F,I,L,V; M208F,W,Y; L217I; V2061 IL,F.

Summary of Invention Paragraph - BSTX (117):

[0113] Still further examples of mutations which appear to be of importance, inter alia, in achieving improved thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): A354C+V479C; L351C+M430C; N457D,E+K385R; L355D,E+M430R,K; L355D,E+I411R,K; and N457D,E.

Summary of Invention Paragraph - BSTX (215):

[0211] .alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 1 can barely be detected. Stabilized variants show, under the same conditions, increased color intensity due to increased

liberation of Cibacron Red.

Summary of Invention Paragraph - BSTX (225):
[0221] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (74):
[0386] Determination of oxidation **stability of M202 substitution variants** of the parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No. 1 and SEQ ID No. 2

Detail Description Paragraph - DETX (75):
[0387] A: Oxidation **stability of variants** of the sequence in SEQ ID No. 1

Detail Description Paragraph - DETX (80):
[0392] B: Oxidation **stability of variants** of the sequence in SEQ ID No. 2

Detail Description Paragraph - DETX (84):
[0395] Determination of thermal **stability of variants** of the parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No. 1 and SEQ ID No. 2

Detail Description Paragraph - DETX (85):
[0396] A: Thermal **stability of pairwise deletion variants** of the sequence in SEQ ID No. 1

Detail Description Paragraph - DETX (93):
[0404] It is apparent that all of the pairwise deletion variants tested exhibit significantly improved thermal stability relative to the parent .alpha.-amylase (SEQ ID No. 1), and that the thermal **stability of Variant** 5,

which in addition to the pairwise deletion mutation of Variant 4 comprises the substitution R124P, is markedly higher than that of the other variants. Since calorimetric results for the substitution variant R124P (comprising only the substitution R124P) reveal an approximately 7.degree. C. thermostabilization thereof relative to the parent .alpha.-amylase, it appears that the thermostabilizing effects of the mutation R124P and the pairwise deletion, respectively, reinforce each other.

Detail Description Paragraph - DETX (94):

[0405] B: Thermal **stability of pairwise deletion variants** of the sequence in SEQ ID No. 2

Detail Description Paragraph - DETX (100):

[0411] C: Thermal **stability of a multi-combination variant** of the sequence in SEQ ID No. 1

PGPUB-DOCUMENT-NUMBER: 20020081670

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020081670 A1

TITLE: Starch debranching enzymes

PUBLICATION-DATE: June 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bisgard-Frantzen, Henrik	Bagsvaerd		DK	
Svendsen, Allan	Birkerod		DK	

APPL-NO: 09/ 833435

DATE FILED: April 12, 2001

RELATED-US-APPL-DATA:

child 09833435 A1 20010412

parent continuation-of 09346237 19990701 US GRANTED

parent-patent 6265197 US

non-provisional-of-provisional 60094353 19980728 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 1998 00868	1998DK-PA 1998 00868	July 2, 1998

US-CL-CURRENT: 435/101, 435/210 , 435/440

ABSTRACT:

The invention relates to a genetically engineered variant of a parent starch debranching enzyme, i.e. a pullulanase or an isamylase, the enzyme variant having an improved thermostability at a pH in the range of 4-6 compared to the parent enzyme and/or an increased activity towards amylopectin and/or glycogen compared to the parent enzyme, to methods for producing such starch debranching enzyme variants with improved thermostability and/or altered substrate specificity, and to a method for converting starch to one or more sugars using at least one such enzyme variant.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 09/346,237, filed Jul. 1, 1999, which claims priority under 35 U.S.C. 119 of Danish application PA 1998 00868, filed Jul. 2, 1998, and the benefit of U.S.

provisional application Ser. No. 60/094,353 filed on Jul. 28, 1998, the contents of which are fully incorporated herein by reference.

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Summary of Invention Paragraph - BSTX (75):
[0072] Examples of specific **.alpha.-amylases** which can be used in the liquefaction step include **Bacillus** licheniformis **.alpha.-amylases**, such as the commercially available products Termamyl.RTM., Spezyme.RTM. AA, Spezyme.RTM. Delta AA, Maxamyl.RTM. and Kleistase.RTM., and the **.alpha.-amylase mutants** described in WO 96/23874 (Novo Nordisk) and PCT/DK97/00197 (Novo Nordisk).

Summary of Invention Paragraph - BSTX (151):
[0148] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent enzyme, wherein the **variant exhibits improved thermal stability** relative to the parent, the method comprising:

Summary of Invention Paragraph - BSTX (154):
[0151] (c) screening for host cells expressing an enzyme **variant which has an altered property (e.g. thermal stability)** relative to the parent enzyme.

PGPUB-DOCUMENT-NUMBER: 20020068352

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020068352 A1

TITLE: Alpha-amylase variants with altered 1, 6-activity

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Svendsen, Allan	Horsholm		DK	
Jorgensen, Christel Thea	Kobenhavn O		DK	
Nielsen, Bjarne Ronfeldt	Virum		DK	

APPL-NO: 09/ 854346

DATE FILED: May 11, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60205229 20000517 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00779	2000DK-PA 2000 00779	May 12, 2000

US-CL-CURRENT: 435/202, 435/183 , 435/195 , 435/69.1 , 510/392 , 510/393

ABSTRACT:

The present invention relates to variants of parent Termamyl-like alpha-amylases, which variant has alpha-amylase activity and exhibits an alteration in the alpha-1,6-D-glucosidic branch linkage cleavage activity of amylopectin and limit dextrins.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims, under 35 U.S.C. 119, priority of Danish application Ser. No. PA 2000 00779 filed May 12, 2000, and the benefit of U.S. provisional application No. 60/205,229, filed May 17, 2000, the contents of which are fully incorporated herein by reference.

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Detail Description Paragraph - DETX (91):

[0121] In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent alpha-amylase, e.g.,

wherein the **variant exhibits alt r d r increased thermal stability** relative to the parent, the method comprising:

Detail Description Paragraph - DETX (94):
[0124] (c) screening for host cells expressing an .sub..alpha.-amylase **variant which has an altered property (i.e. thermal stability)** relative to the parent .sub..alpha.-amylase.

Detail Description Paragraph - DETX (114):
[0141] In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **alpha-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amylolique-faciens **alpha-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **alpha-amylase,** A. niger acid stable **alpha-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detail Description Paragraph - DETX (164):
[0179] 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed

Detail Description Paragraph - DETX (183):
[0194] The following **variants** are constructed as described in EXAMPLE 1 of WO 00/29560 (from Novozymes A/S) in the **Bacillus** licheniformis **alpha-amylase** shown in SEQID NO: 8:

Claims Text - CLTX (4):
4. The **variant** of any of claims 1-3, wherein the parent Termamyl-like **alpha-amylase** is derived from a strain of B. licheniformis, B. amyloliquefaciens, B. stearothermophilus, **Bacillus** sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, or DSMZ no. 12649, KSM AP1378.

PGPUB-DOCUMENT-NUMBER: 20020037824

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020037824 A1

TITLE: Detergent compositions comprising a maltogenic alpha-amylase enzyme and a detergent ingredient

PUBLICATION-DATE: March 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Smets, Johan	Lubbeek		BE	
Pintens, An	Merksem		BE	

APPL-NO: 09/ 888758

DATE FILED: June 25, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	PCT/US00/18120	2000US-PCT/US00/18120	June 30, 2000

US-CL-CURRENT: 510/305, 510/306 , 510/313 , 510/392

ABSTRACT:

The present invention relates to detergent compositions, including laundry, dishwashing, and/or hard surface cleaner compositions, comprising a maltogenic alpha-amylase enzyme and a detergent ingredient selected from the group consisting of a nonionic surfactant, a protease, a bleaching agent and/or mixtures thereof. Such compositions provide excellent removal of starch-containing stains and soils, and when formulated as laundry compositions, excellent whiteness maintenance and dingy cleaning.

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Summary of Invention Paragraph - BSTX (12):

[0010] Maltogenic **alpha-amylases** are known for various industrial applications. In particular, this enzyme finds potential application for retarding or preventing retrogradation and thus the staling of starch based common food as common in the baking industry. These enzymes are useful as well in the process for the manufacture of linear oligosaccharides or in the production of sweeteners and ethanol from starch and/or textile industry. A maltogenic **alpha-amylase from Bacillus** (EP 120 693) is commercially available under the tradename Novamyl.RTM. (Product of Novo Nordisk A/S, Denmark) and is widely used in the baking industry as an anti-staling agent due to its ability to reduce retrogradation of starch. WO99/43794 describes **variants** of

maltogenic **alpha-amylases** with improved properties : altered pH optimum, improved thermostability, increased specific activity, altered cleavage pattern or increased ability to educe retrogradation of starch or staling of bread. WO99/43793 discloses **variants** of maltogenic **alpha-amylases** having CGT-ase activity, CGT-ase having maltogenic **alpha-amylase** activity and hybrid enzymes with one or more improvements to the specific properties of the parents enzymes.

Detail Description Paragraph - DETX (15):

[0026] In more details, WO99/43793 provides for **variants** of maltogenic **alpha-amylase** and CGT-ase and hybrids wherein the parent maltogenic **alpha-amylase** used in the invention is an enzyme classified in EC 3.2.1.133, preferably maltogenic **alpha-amylase** used, is the amylase cloned from **Bacillus** as described in EP 120 693 and wherein the parent CGT-ase used is an enzyme classified in EC 2.4.1.19. and has preferably one or more of the following characteristics:

Detail Description Paragraph - DETX (44):

[0055] The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimise their performance efficiency in the detergent compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant **stability, catalytic activity and the like, of the enzyme variant** is tailored to suit the particular cleaning application.

Detail Description Paragraph - DETX (74):

[0084] A second essential element of the detergent compositions of the present invention can be a protease enzyme. As mentioned above, the starch containing stains and soils comprise many proteins components as well. Without wishing to be bound by theory it is believed that the protease enzyme hydrolyses the proteins contains in such complex stains and thereby induces the synergistic removal of such stains/soils with the maltogenic **alpha-amylase**. In addition, such hydrolysed complex stains/soils have a lower molecular weight in the wash solution and therefore it results in less redeposition of such hydrolysed complex stains on the surface to be cleaned. Suitable proteases are the subtilisins which are obtained from particular strains of B. subtilis and B. licheniformis (subtilisin BPN and BPN'). One suitable protease is obtained from a strain of **Bacillus**, having maximum activity throughout the pH range of 8-12, developed and sold as ESPERASE.RTM. by Novo Industries A/S of Denmark, hereinafter "Novo". The preparation of this enzyme and analogous enzymes is described in GB 1,243,784 to Novo. Other suitable proteases include ALCALASE.RTM., DURAZYM.RTM. and SAVINASE.RTM. (protease Subtilisin 309 from

Bacillius subtilis) from Novo and MAXATASE.RTM., MAXACAL.RTM., PROPERASE.RTM. and MAXAPEM.RTM. (protein engineered Maxacal) from Gist-Brocades. Also suitable for the present invention are proteases described in patent applications EP 251 446 and WO 91/06637, protease BLAP.RTM. described in WO91/02792 and their **variants** described in WO 95/23221. See also a high pH protease from **Bacillus** sp. NCIMB 40338 described in WO 93/18140 A to Novo. Enzymatic detergents comprising protease, one or more other enzymes, and a reversible protease inhibitor are described in WO 92/03529 A to Novo. When desired, a protease having decreased adsorption and increased hydrolysis is available as described in WO 95/07791 to Procter & Gamble. A recombinant trypsin-like protease for detergents suitable herein is described in WO 94/25583 to Novo. Other suitable proteases are described in EP 516 200 by Unilever.

Detail Description Paragraph - DETX (200):

[0208] As indicated above, the detergent compositions of the present invention will preferably comprise an .alpha.-amylase. Suitable .alpha.-amylases for the purpose of the present invention are described in the following : WO94/02597, Novo Nordisk A/S published Feb. 03, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO95/10603, Novo Nordisk A/S, published Apr. 20, 1995. Other amylases known for use in cleaning compositions include both .alpha.-and .beta.-amylases. (.alpha.-Amylases are known in the art and include those disclosed in U.S. Pat. No. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314, published Aug. 18, 1994 and WO96/05295, Genencor, published Feb. 22, 1996 and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95. Also suitable are amylases described in EP 277 216, WO95/26397 and WO96/23873 (all by Novo Nordisk). Examples of commercial .alpha.-amylases products are Purafect Ox Am.RTM. from Genencor and Termamyl.RTM., Ban.RTM., Fungamyl.RTM. and Duramyl.RTM., all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases: .alpha.-amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl.RTM. at a temperature range of 25.degree. C. to 55.degree. C. and at a pH value in the range of 8 to 10, measured by the Phadebas.RTM. .alpha.-amylase activity assay. Preferred are variants of the above enzymes, described in WO96/23873 (Novo Nordisk). Preferably, the **variants are those demonstrating improved thermal stability,** more preferably those wherein at least one amino acid residue equivalent to F180, R181, G182, T183, G184, or K185 has been deleted from the parent .alpha.-amylase. Particularly preferred are those variants having improved thermal stabilitywhich comprise the amino acid deletions R181.sup.*+G182.sup.* or T183.sup.*+G184.sup.*. Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermal stability and a higher activity level are described in WO95/35382. Further suitable amylases are the H mutant .alpha.-amylase enzymes exhibiting improved stability described in WO98/26078 by Genencor.

Detail Description Paragraph - DETX (218):

[0226] The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimise their performance efficiency in the detergent compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant **stability, catalytic activity and the like, of the enzyme variant** is tailored to suit the particular cleaning application.

Detail Description Table CWU - DETL (1):

1 LAS: Sodium linear C.sub.11-13 alkyl benzene sulphonate. TAS: Sodium tallow alkyl sulphate. OxyAS: Sodium C.sub.1x-C.sub.1y alkyl sulfate. OxySAS: Sodium C.sub.1x-C.sub.1y secondary (2,3) alkyl sulfate. CxyEz: C.sub.1x-C.sub.1y predominantly linear primary alcohol condensed with an average of z moles of ethylene oxide. CxyEzS: C.sub.1x-C.sub.1y sodium alkyl sulfate condensed with an average of z moles of ethylene oxide. CxEOy: Cy alcohol with an average of ethoxylation of y. NI 1: Mixed ethoxylated/propoxylated fatty alcohol e.g. Plurafac LF404 being an alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5. NI 2: C12-C14 alkyldimethyl amine oxide QAS: R.sub.2.N+(CH.sub.3).sub.2(- C.sub.2H.sub.4OH) with R.sub.2 = C.sub.12-C.sub.14. QAS 1: R.sub.2.N+(CH.sub.3).sub.2(C.sub.2H.sub.4OH) with R.sub.2 = C.sub.8-C.sub.11. SADS: Sodium C14-22 alkyl disulphate of fromula 2-(R).C4H7- 1,4-(SO4-)2 where R = C10-18 MBAS: C12-18 mid branched alkyl sulphate surfactant with an average branching of 1.5 methyl or ethyl branching groups MES: x-Sulpho methylester of C18 fatty acid APA: C.sub.8-10 amido propyl dimethyl amine. Soap: Sodium linear alkyl carboxylate derived from a 80/20 mixture of tallow and coconut fatty acids. STS: Sodium toluene sulphonate. TFAA: C.sub.16-C.sub.18 alkyl N-methyl glucamide. TPKFA: C.sub.12-C.sub.14 topped whole cut fatty acids. DEQA: Di-(tallow-oxy-ethyl) dimethyl ammonium chloride. DEQA (2): Di-(soft-tallowyloxyethyl) hydroxyethyl methyl ammonium methylsulfate. SDASA: 1:2 ratio of stearyldimethyl amine:triple-pressed stearic acid. DTMAMS: Ditallow dimethyl ammonium methylsulfate. Silicate: Amorphous Sodium Silicate (SiO.sub.2:Na.sub.2O ratio = 1.6- 3.2:1). Metasilicate: Sodium metasilicate (SiO.sub.2:Na.sub.2O ratio = 1.0). Zeolite A: Hydrated Sodium Aluminosilicate of formula Na.sub.12(A1O.sub.2SiO.sub.2).sub.12.27H.sub.2O having a primary particle size in the range from 0.1 to 10 micrometers (Weight expressed on an anhydrous basis). SKS-6: Crystalline layered silicate of formula .delta.-Na.sub.2Si.sub.2O.sub.5 Citrate: Tri-sodium citrate dihydrate. Citric: Anhydrous citric acid. Carbonate: Anhydrous sodium carbonate. Bicarbonate: Sodium hydrogen carbonate. Sulphate: Anhydrous sodium sulphate. Mg Sulphate: Anhydrous magnesium sulfate. STPP: Sodium tripolyphosphate. TSPP: Tetrasodium pyrophosphate. MNAA: Random copolymer of 4:1 acrylate/maleate, average molecular weight about 70,000-80,000. MA/AA 1: Random copolymer of 6:4 acrylate/maleate, average molecular weight about 10,000. AA: Sodium polyacrylate polymer of average molecular weight 4,500.

Polycarboxylate: Copolymer comprising mixture of carboxylated monomers such as acrylate, maleate and methyacrylate with a MW ranging between 2,000-80,000 such as Sokolan commercially available from BASE, being a copolymer of acrylic acid, MW 4,500. Clay: Bentonite or smectite clay. PB1: Anhydrous sodium perborate monohydrate. PB4: Sodium perborate tetrahydrate of nominal formula NaBO.sub.3.4H.sub.2O. Percarbonate: Anhydrous sodium percarbonate of nominal formula Na.sub.2CO.sub.3.3H.sub.2O.sub.2. NaDOC: Sodium dichloroisocyanurate. TAED: Tetraacetyl ethylene diamine. NOBS: Nonanoyloxybenzene sulfonate in the form of the sodium salt. NACA-OBS: (6-nonamidocaproyl) oxybenzene sulfonate. LOBS: Dodecanoyloxybenzene sulfonate in the form of the Na salt. DOBA: Dodecanoylbenzoic acid DTPA: Diethylene triamine pentaacetic acid. HEDP: 1,1-hydroxyethane diphosphonic acid. DETPMP: Diethyltriamine penta (methylene) phosphonate, marketed by Monsanto under the Trade name Dequest 2060. EDDS: Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer in the form of its sodium salt MnTACN: Manganese 1,4,7-trimethyl-1,4,7-triazacycl- ononane. Photoactivated: Sulfonated zinc or alumino phtalocyanine encapsulated Bleach in dextrin soluble polymer. PAAC: Pentaamine acetate cobalt (III) salt. Paraffin: Paraffin oil sold under the tradename Winog 70 by Wintershall. NaBz: Sodium benzoate. Protease: Proteolytic enzyme sold under the tradename Savinase by Novo Nordisk A/S, the "Protease B" variant with the substitution Y217L described in EP 251 446, the "protease D" variant with the substitution set N76D/S103A/V104I and the protease described in WO99/20727, WO99/20726 and WO99/20723 with the amino acid substitution set 101G/103A/1041/159D/232V/- 236H/245R/248D/252K. Amylase: Amylolytic enzyme sold under the tradename Termamyl .RTM. and Duramyl .RTM. available from Novo Nordisk A/S and those **variants having improved thermal stability** with amino acid deletions P181* + G182* or Ti 83* + G184* as described in WO95/35382. Lipase: Lipolytic enzyme sold under the tradename Lipolase, Lipolase Ultra by Novo Nordisk A/S and Lipomax by Gist-Brocades. MaltoH: Maltogenic alpha-amylase sold under the tradename Novamyl by Novo Nordisk A/S AMG: Amyloglucosidase sold under the tradename AMG from Novo Nordisk A/S. Cellulase: Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novo Nordisk A/S. CMC: Sodium carboxymethyl cellulose. PVP: Polyvinyl polymer, with an average molecular weight of 60,000. PVNO: Polyvinylpyridine-N-Oxide, with an average molecular weight of 50,000. PVPVI: Copolymer of vinylimidazole and vinylpyrrolidone, with an average molecular weight of 20,000. Brightener 1: Disodium 4,4'-bis(2-sulphostyryl)biphenyl. Brightener 2: Disodium 4,4'-bis(4-anilino-6-morpholino-1,3,5-triazin-2- yl)stilbene-2:2'-disulfonate. Brightener 3: Disodium 4,4'bis(4,6-dianilino-1,3,5-triazin-2-yl)amino stilbene-2-2'-disulfonate. Silicone antifoam: Polydimethylsiloxane foam controller with siloxane- oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1. Suds Suppressor: 12% Silicone/silica, 18% stearyl alcohol,70% starch in granular form. Thickener: High molecular weight crosslinked polyacrylates such as Carbopol offered by B.F. Goodrich Chemical Company and Polygel. SRP 1: Anionically end capped poly esters. SRP 2: Soil Release Polymer selected from 1) Non-cotton soil release polymer according to U.S. Pat. No. 5,415,807, Gosselink, Pan, Kellett and Hall, issued May 16, 1995 or and/or from 2) Non-cotton soil release polymer according to US application no. 60/051517. QEA: bis((C.sub.2H.sub.5O)(C.sub.2H.sub.4O).sub.n)(CH.s- ub.3) --N.sup.+-C.sub.6H.sub.12--N.sup.+-(CH.sub.3) bis((C.sub.2H.sub.5O)(C.sub.2H.sub.4O)).sub.n, wherein n = from 20 to 30. PEI:

Polyethyleneimine with an average molecular weight of between 600-1800 and an average ethoxylation degree of 7-20 ethyleneoxy residues per nitrogen. SCS: Sodium cumene sulphonate. HMWPEO: High molecular weight polyethylene oxide. PEG X: Polyethylene glycol, of a molecular weight of X PEO: Polyethylene oxide, with an average molecular weight of 5,000. TEPAE: Tetreaethylenepentaamine ethoxylate. BTA: Benzotriazole. PH: Measured as a 1% solution in distilled water at 20.degree. C.

PGPUB-DOCUMENT-NUMBER: 20020032142

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020032142 A1

TITLE: Detergent compositions comprising a cyclodextrin glucanotransferase enzyme and a detergent ingredient

PUBLICATION-DATE: March 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Smets, Johan	Lubbeek		BE	
Pintens, An	Merksem		BE	

APPL-NO: 09/ 888714

DATE FILED: June 25, 2001

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	PCT/US00/18119	2000US-PCT/US00/18119	June 30, 2000

US-CL-CURRENT: 510/305, 510/311 , 510/312 , 510/392

ABSTRACT:

The present invention relates to detergent compositions, including laundry, dishwashing, and/or hard surface cleaner compositions, comprising a cyclodextrin glucanotransferase enzyme and a detergent ingredient selected from a nonionic surfactant, a protease and/or a bleaching agent. Such compositions provide excellent removal of starch-containing stains and soils and malodor control; and when formulated as laundry compositions, excellent whiteness maintenance and dingy cleaning.

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Summary of Invention Paragraph - BSTX (148):

[0144] In more details, WO99/43793 provides for **variants** of maltogenic **alpha-amylase** and CGT-ase and hybrids wherein the parent maltogenic **alpha-amylase** used in the invention is an enzyme classified in EC 3.2.1.133, preferably maltogenic **alpha-amylase** used, is the amylase cloned from **Bacillus** as described in EP 120 693 and wherein the parent CGT-ase used is an enzyme classified in EC 2.4.1.19. and has preferably one or more of the following characteristics:

Summary of Invention Paragraph - BSTX (162):

[0158] The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimise their performance efficiency in the detergent compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant **stability, catalytic activity and the like, of the enzyme variant** is tailored to suit the particular cleaning application.

Summary of Invention Paragraph - BSTX (299):

[0292] As indicated above, the detergent compositions of the present invention will preferably comprise an (x-amylase. Suitable .alpha.-amylases for the purpose of the present invention are described in the following : WO94/02597, Novo Nordisk A/S published Feb. 03, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO95/10603, Novo Nordisk A/S, published Apr. 20, 1995. Other amylases known for use in cleaning compositions include both .alpha.- and .beta.-amylases. .alpha.-Amylases are known in the art and include those disclosed in U.S. Pat. No. 5,003,257; EP 252,666; WO/91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent specification no. 1,296,839 (Novo). Other suitable amylases are stability-enhanced amylases described in WO94/18314, published Aug. 18, 1994 and WO96/05295, Genencor, published Feb. 22, 1996 and amylase variants having additional modification in the immediate parent available from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95. Also suitable are amylases described in EP 277 216, WO95/26397 and WO96/23873 (all by Novo Nordisk). Examples of commercial (x-amylases products are Purafect Ox Am.RTM. from Genencor and Termamyl.RTM., Ban.RTM., Fungamyl.RTM. and Duramyl.RTM., all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases: (.alpha.-amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl.RTM. at a temperature range of 25.degree. C. to 55.degree. C. and at a pH value in the range of 8 to 10, measured by the Phadebas.RTM. .beta.-amylase activity assay. Preferred are variants of the above enzymes, described in WO96/23873 (Novo Nordisk). Preferably, the **variants are those demonstrating improved thermal stability,** more preferably those wherein at least one amino acid residue equivalent to F180, R181, G182, T183, G184, or K185 has been deleted from the parent .alpha.-amylase. Particularly preferred are those **variants having improved thermal stability** which comprise the amino acid deletions R181 +G182 or T183 +G184*. Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermal stability and a higher activity level are described in WO95/35382. Further suitable amylases are the H mutant .alpha.-amylase enzymes exhibiting improved stability described in WO98/26078 by Genencor.

Summary of Invention Paragraph - BSTX (311):

[0304] The above-mentioned enzymes may be of any suitable origin, such as

vegetable, animal, bacterial, fungal and yeast origin. Origin can further be mesophilic or extremophilic (psychrophilic, psychrotrophic, thermophilic, barophilic, alkalophilic, acidophilic, halophilic, etc.). Purified or non-purified forms of these enzymes may be used. Nowadays, it is common practice to modify wild-type enzymes via protein/genetic engineering techniques in order to optimise their performance efficiency in the detergent compositions of the invention. For example, the variants may be designed such that the compatibility of the enzyme to commonly encountered ingredients of such compositions is increased. Alternatively, the variant may be designed such that the optimal pH, bleach or chelant **stability, catalytic activity and the like, of the enzyme variant** is tailored to suit the particular cleaning application.

PGPUB-DOCUMENT-NUMBER: 20020010122

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020010122 A1

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

PUBLICATION-DATE: January 24, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Wyoming	OH	US	
Meyer, Axel	Cincinnati	OH	US	

APPL-NO: 09/ 789884

DATE FILED: February 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60184268 20000223 US

US-CL-CURRENT: 510/374, 510/499 , 510/504

ABSTRACT:

The present invention relates to liquid laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising:
a) from about 0.01 to about 20% by weight, of a zwitterionic polymer which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise an anionic moiety is less than or equal to the number of quaternized backbone amino units;
b) from about 0. 1% to about 7% by weight, of a polyamine dispersant;
c) from about 0.01% to about 80% by weight, of a surfactant system comprising one or more surfactants selected from the group consisting of nonionic, anionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof; and
d) the balance carriers and adjunct ingredients.

CROSS REFERENCE

[0001] This Application claims the benefit of U.S. Provisional Application No. 60/184,268, filed on Feb. 23, 2000.

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Detail Description Paragraph - DETX (79):
[0169] Derivatives of Bacillus amyloliquefaciens Subtilisin --BPN' enzymes A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detail Description Paragraph - DETX (81):
[0171] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detail Description Paragraph - DETX (108):
[0197] Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, Jun. 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative

stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, March 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

PGPUB-DOCUMENT-NUMBER: 20020006647

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020006647 A1

TITLE: Fermentation with a phytase

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Veit, Chris	Wake Forest	NC	US	
Felby, Claus	Herlev	NC	DK	
Peckous, Larry W.	Raleigh		US	
Olsen, Hans Sejr	Holte		DK	

APPL-NO: 09/ 788906

DATE FILED: February 20, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60185716 20000223 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
DK	PA 2000 00281	2000DK-PA 2000 00281	February 23, 2000

US-CL-CURRENT: 435/162

ABSTRACT:

The present invention relates to an improved fermenation process wherein phytic acid-containing material is fermented in the presence of a phytase.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. 119 of Danish application PA 2000 00281 filed Feb. 23, 2000 and U.S. Provisional application Ser. No. 60/185,716, filed Feb. 23, 2000, the contents of which are fully incorporated herein by reference.

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Detail Description Paragraph - DETX (66):

[0072] The liquefaction step may be performed in the presence of an **alpha-amylase** derived from a microorganism or a plant. Preferred **alpha-amylases** are of fungal or bacterial origin. **Bacillus alpha-amylases**

(often referred to as "Termamyl-like **alpha-amylases"), variant** and hybrids thereof, are specifically contemplated according to the invention. Well-known Termamyl-like **alpha-amylases** include **alpha-amylase** derived from a strain of B. licheniformis (commercially available as Termamyl.TM.) B. amyloliquefaciens, and B. stearothermophilus **alpha-amylase** (BSG). Other Termamyl-like **alpha-amylases** include **alpha-amylase** derived from a strain of the **Bacillus** sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375, all of which are described in detail in WO 95/26397, and the **alpha-amylase** described by Tsukamoto et al., Biochemical and Biophysical Research Communications, 151 (1988), pp. 25-31. In the context of the present invention, a Termamyl-like **alpha-amylase is an alpha-amylase** as defined in WO 99/19467 on page 3, line 18 to page 6, line 27. Contemplated **variants** and hybrids are described in WO 96/23874, WO 97/41213, and WO 99/19467. Contemplated **alpha-amylase** derived from a strain of Aspergillus includes Aspergillus oryzae and Aspergillus niger **alpha-amylases**. Commercial **alpha-amylase** products and products containing **alpha-amylases** include TERMAMYL.TM. SC, FUNGAMYL.TM., LIQUOZYME.TM. and SAN.TM. SUPER.

Detail Description Paragraph - DETX (71):

[0077] Other suitable Aspergillus glucoamylase **variants include variants to enhance the thermal stability**: G137A and G139A (Chen et al. (1996), Prot. Engng. 9, 499-505); D257E and D293E/Q (Chen et al. (1995), Prot. Engng. 8, 575-582); N182 (Chen et al. (1994), Biochem. J. 301, 275-281); disulphide bonds, A246C (Fierobe et al. (1996), Biochemistry, 35, 8698-8704; and introduction of Pro residues in position A435 and S436 (Li et al. (1997), Protein Engng. 10, 1199-1204. Furthermore Clark Ford presented a paper on Oct 17, 1997, ENZYME ENGINEERING 14, Beijing/China Oct 12-17, 97, Abstract number: Abstract book p. 0-61. The abstract suggests mutations in positions G137A, N20C/A27C, and S30P in an Aspergillus awamori glucoamylase to improve the thermal stability.

PGPUB-DOCUMENT-NUMBER: 20020004474

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020004474 A1

TITLE: Laundry detergent compositons comprising hydrophobically modified polyamines and nonionic surfactants

PUBLICATION-DATE: January 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Price, Kenneth Nathan	Cincinnati	OH	US	

APPL-NO: 09/ 790042

DATE FILED: February 21, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60184250 20000223 US

US-CL-CURRENT: 510/375, 510/376 , 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions comprising: A) from about 0.01%, preferably from about 0.1%, more preferably from about 1%, most preferably from about 3% to about 50%, preferably to about 20%, more preferably to about 10%, most preferably to about 7% by weight, of a hydrophobically modified polyamine having the formula: 1
wherein R is C.sub.5-C.sub.20 linear or branched alkylene, and mixtures thereof; R.sup.1 is an alkyleneoxy unit having the formula:
--(R.sup.2O).sub.x--R.sup.3
wherein R.sup.2 is C.sub.2-C.sub.4 linear or branched alkylene, and mixtures thereof; R.sup.3 is hydrogen, C.sub.1-C.sub.22 alkyl, C.sub.7-C.sub.22 alkylenearyl, an anionic unit, and mixtures thereof; x is from about 15 to about 30; Q is a hydrophobic quaternizing unit selected from the group consisting of C.sub.8-C.sub.30 linear or branched alkyl, C.sub.6-C.sub.30 cycloalkyl, C.sub.7-C.sub.30 substituted or unsubstituted alkylenearyl, and mixtures thereof; X is an anion present in sufficient amount to provide electronic neutrality; n is from 0 to 4;
B) from about 0.01% by weight, of a surfactant system comprising one or more surfactants selected from:
i) from about 85% to about 99.9% by weight, of one or more nonionic surfactants;
ii) optionally, from about 0.1% to about 15% by weight, of one or more anionic surfactants;
iii) optionally from about 0.1% to about 15% by weight, of one or more cationic

surfactants;
iv) optionally from about 0.1% to about 15% by weight, of one or more zwitterionic surfactants;
v) optionally from about 0.1% to about 15% by weight, of one or more ampholytic surfactants; or
vi) mixtures thereof;
C) the balance carriers and adjunct ingredients.

CROSS-REFERENCE

[0001] This Application claims the benefit of U.S. Provisional Application no. 60/184,250, filed on Feb. 23, 2000.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (178):
[0162] A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Summary of Invention Paragraph - BSTX (180):
[0163] A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Summary of Invention Paragraph - BSTX (205):
[0182] Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in

commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCE38061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6579839

DOCUMENT-IDENTIFIER: US 6579839 B2

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

DATE-ISSUED: June 17, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Kenneth Nathan	Wyoming	OH	N/A	N/A
Meyer; Axel	Cincinnati	OH	N/A	N/A

APPL-NO: 10/ 284665

DATE FILED: October 31, 2002

PARENT-CASE:

CROSS REFERENCE

This application is a con't of Ser. No. 09/789,884, filed Feb. 21, 2001 now U.S. Pat. No. 6,525,012, which claims priority under 35 USC 119(e) to provisional application No. 60/184,268, filed Feb. 23, 2000.

US-CL-CURRENT: 510/321, 510/338 , 510/340 , 510/351 , 510/356 , 510/357 , 510/360 , 510/393 , 510/504 , 510/530

ABSTRACT:

The present invention relates to liquid laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising: a) from about 0.01 to about 20% by weight, of a zwitterionic polymer which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise an anionic moiety is less than or equal to the number of quaternized backbone amino units; b) from about 0.1% to about 7% by weight, of a polyamine dispersant; c) from about 0.01% to about 80% by weight, of a surfactant system comprising one or more surfactants selected from the group consisting of nonionic, anionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof; and d) the balance carriers and adjunct ingredients.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (94):

A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gin, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detailed Description Text - DETX (96):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (124):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative

stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510663 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6541207

DOCUMENT-IDENTIFIER: US 6541207 B1

TITLE: Methods for generating recombined polynucleotides

DATE-ISSUED: April 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vind; Jesper	V.ae butted.rl.o	N/A	N/A	DK
Borchert; Torben Vedel	slashed.se .O slashed.sterbro	N/A	N/A	DK

APPL-NO: 09/ 687301

DATE FILED: October 13, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Rule 1.53(b) continuation application of U.S. patent application Ser. No. 09/040,697, filed Mar. 18, 1998, now U.S. Pat. No. 6,159,687 issued Dec. 12, 2000.

This application claims priority under 35 U.S.C. 119 of Danish applications 0307/97 filed Mar. 18, 1997, 0434/97 filed Apr. 17, 1997, and 0625/97 filed May 30, 1997, and U.S. Provisional applications Ser. Nos. 60/044,836, filed Apr. 25, 1997 and Ser. No. 60/153,012 filed Jun. 24, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0307/97	March 18, 1997
DK	0625/97	May 30, 1997
DK	0434/97	April 17, 1997

US-CL-CURRENT: 435/6, 435/4 , 435/455 , 435/468 , 435/471 , 435/69.1 , 435/91.1 , 435/91.2 , 536/23.1

ABSTRACT:

A method for in vitro construction of a library of recombined homologous polynucleotides from a number of different starting DNA templates and primers by induced template shifts during an polynucleotide synthesis is described, whereby A. extended primers are synthesized by a) denaturing the DNA templates b) annealing primers to the templates, c) extending the said primers by use of a polymerase, d) stop the synthesis, and e) separate the extended primers from the templates, B. a template shift is induced by a) isolating the extended

primers from the templates and repeating steps A.b) to A.e) using the extended primers as both primers and templates, or b) repeating steps A.b) to A.e), C. this process is terminated after an appropriate number of cycles of process steps A. and B.a), A. and B.b), or combinations thereof.

Optionally the polynucleotides are amplified in a standard PCR reaction with specific primers to selectively amplify homologous polynucleotides of interest.

30 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (101):
Strauberg et al. (Biotechnology 13: 669-673 (1995) describes a screening system for subtilisin **variants having Calcium-independent stability**;

Detailed Description Text - DETX (40):
In Example 1, it was shown how a number of multiple **variants** of H. lanuginosa lipase were shuffled. In a similar manner, **variants of Bacillus .alpha.-amylases** can be shuffled.

Detailed Description Text - DETX (41):
Earlier patent applications have identified **variants of various .alpha.-amylases from Bacillus** species improved for particular properties, e.g. thermostability, stability under Calcium-depleted conditions, improved wash-performance etc. (see WO95/10603, WO96/23874, WO96/23873, and PCT/DK97/00197).

US-PAT-NO: 6528298

DOCUMENT-IDENTIFIER: US 6528298 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: March 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerod	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen	N/A	N/A	DK
Bisgard-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK
Outtrup; Helle	Ballerup	N/A	N/A	DK
Nielsen; Bjarne Ronfeldt	Virum	N/A	N/A	DK
Nielsen; Vibeke Skovgaard	Bagsv.oe butted.rd	N/A	N/A	DK
Hedegaard; Lisbeth	Skodsborg	N/A	N/A	DK

APPL-NO: 09/ 545586

DATE FILED: April 7, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of application Ser. No. 09/290,734 filed on Apr. 13, 1999, now U.S. Pat. No. 6,361,989, issued Mar. 26, 2002 which is a continuation-in-part of application Ser. No. 09/170,670 filed on Oct. 13, 1998, now U.S. Pat. No. 6,187,576, issued Feb. 13, 2001 and claims priority under 35 U.S.C. 119 of Danish application no. 1172/97 filed on Oct. 13, 1997, U.S. application Ser. No. 60/063,306, filed on Oct. 28, 1997, and Danish application no. PA 1999 00439 filed on Mar. 31, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1172/97	October 13, 1997
DK	1999 00439	March 31, 1999

US-CL-CURRENT: 435/202, 435/183 , 435/200 , 435/201 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2 , 536/23.7

ABSTRACT:

The invention relates to a novel Termamyl-like .alpha.-amylase, and Termamyl-like .alpha.-amylases comprising mutations in two, three, four, five or six regions/positions. The variants have increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an

.alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent).

12 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

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Brief Summary Text - BSTX (6):

Among more recent disclosures relating to **.alpha.-amylases**, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Detailed Description Text - DETX (177):

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent .alpha.-amylase, e.g. wherein the **variant exhibits altered or increased thermal stability** relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing an .alpha.-amylase **variant which has an altered property (i.e. thermal stability**) relative to the parent .alpha.-amylase.

Detailed Description Text - DETX (197):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows

transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliguefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (231):

Amylases: Suitable amylases (.alpha.- and/or .beta.-) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, .alpha.-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839.

Detailed Description Text - DETX (274):

Enzymes SP690: .alpha.-amylase shown in SEQ ID NO: 1 SP722: .alpha.-amylase shown in SEQ ID NO: 2 Termamyl.RTM.: .alpha.-amylase from Bacillus licheniformis shown in SEQ ID NO: 4. AA560: .alpha.-amylase of the invention shown in SEQ ID NO: 24 encoded by the DNA sequence shown in SEQ ID NO: 23. AA360: .alpha.-amylase shown in SEQ ID NO: 26 being identical to the AA560 .alpha.-amylase encoded by the DNA sequence shown in SEQ ID NO: 25. BSG alpha-amylase: B. stearothermophilus alpha-amylase depicted in SEQ ID NO: 3. TVB146 alpha-amylase variant: B. stearothermophilus alpha-amylase variant depicted in SEQ ID NO: 3.with the following mutations: with the deletion in positions I181-G182+N193F.

Detailed Description Text - DETX (275):

LE174 Hybrid Alpha-amylase Variant LE174 is a hybrid Termamyl-like alpha-amylase being identical to the Termamyl sequence, i.e., the Bacillus licheniformis .alpha.-amylase shown in SEQ ID NO: 4, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the Bacillus amyloliquefaciens alpha-amylase shown in SEQ ID NO: 5, which further have the following mutations: H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 4). LE174 was constructed by SOE-PCR (Higuchi et al. 1988, Nucleic Acids Research 16:7351).

US-PAT-NO: 6525012

DOCUMENT-IDENTIFIER: US 6525012 B2

TITLE: Liquid laundry detergent compositions having enhanced clay removal benefits

DATE-ISSUED: February 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Kenneth Nathan	Wyoming	OH	N/A	N/A
Meyer; Axel	Cincinnati	OH	N/A	N/A

APPL-NO: 09/ 789884

DATE FILED: February 21, 2001

PARENT-CASE:

CROSS REFERENCE

This Application claims the benefit of U.S. Provisional Application No. 60/184,268, filed on Feb. 23, 2000.

US-CL-CURRENT: 510/321, 510/337 , 510/338 , 510/340 , 510/393 , 510/499 , 510/504 , 564/281 , 564/290 , 564/295

ABSTRACT:

The present invention relates to liquid laundry detergent compositions which provide enhance hydrophilic soil cleaning benefits, said compositions comprising: a) from about 0.01 to about 20% by weight, of a zwitterionic polymer which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise an anionic moiety is less than or equal to the number of quaternized backbone amino units; b) from about 0.1% to about 7% by weight, of a polyamine dispersant; c) from about 0.01% to about 80% by weight, of a surfactant system comprising one or more surfactants selected from the group consisting of nonionic, anionic, cationic, zwitterionic, ampholytic surfactants, and mixtures thereof; and d) the balance carriers and adjunct ingredients.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (49):

Derivatives of Bacillus amyloliquefaciens Subtilisin --BPN' enzymes A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detailed Description Text - DETX (51):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (78):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, Jun. 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative

stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, March 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6518054

DOCUMENT-IDENTIFIER: US 6518054 B1

TITLE: Metallo-endopeptidases

DATE-ISSUED: February 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Van den Burg; Lambertus	Epe	N/A	N/A	NL
Veltman; Oene Robert	Groningen	N/A	N/A	NL
Venema; Gerard	Haren	N/A	N/A	NL

APPL-NO: 09/ 381982

DATE FILED: December 7, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	97200931	March 27, 1997

PCT-DATA:
APPL-NO: PCT/NL98/00164
DATE-FILED: March 23, 1998
PUB-NO: WO98/44127
PUB-DATE: Oct 8, 1998
371-DATE: Dec 7, 1999
102(E)-DATE:Dec 7, 1999

US-CL-CURRENT: 435/219, 435/183 , 435/212 , 435/221 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention provides genes encoding variants of metallo-endopeptidases that have been engineered to be resistant to prolonged boiling while having maintained their enzymatic performance at much lower temperatures. In addition, thermal stability of the metallo-endopeptidases is highly dependent on calcium at concentrations in the mM range. The invention further provides active metallo-endopeptidases **variants whose stability** depending on calcium concentration can be changed so as to provide metallo-endopeptidases that are calcium dependent or independent. The invention also provides genes that encode boiling-resistant metallo-endopetidases whose stability depending on calcium concentration can be changed. The invention also provides vectors and cells comprising these genes and proteases produced through these genes, vectors and/or cells. In particular variants with the above described properties are provided of thermolysin-like proteases such as produced by Bacillus stearothermophilus (TLP-ste) and Bacillus thermoproteolyticus (thermolysine). Boiling-resistant

and calcium independent or dependent metallo-endopeptidases can be applied in several industrial processes, for instance in the preparation of the artificial sweetener aspartame, but also in the leather industry, in breweries and in the production of protein hydrolysates.

13 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Abstract Text - ABTX (1):

The present invention provides genes encoding variants of metallo-endopeptidases that have been engineered to be resistant to prolonged boiling while having maintained their enzymatic performance at much lower temperatures. In addition, thermal stability of the metallo-endopeptidases is highly dependent on calcium at concentrations in the mM range. The invention further provides active metallo-endopeptidases **variants whose stability** depending on calcium concentration can be changed so as to provide metallo-endopeptidases that are calcium dependent or independent. The invention also provides genes that encode boiling-resistant metallo-endopetidases whose stability depending on calcium concentration can be changed. The invention also provides vectors and cells comprising these genes and proteases produced through these genes, vectors and/or cells. In particular variants with the above described properties are provided of thermolysin-like proteases such as produced by Bacillus stearothermophilus (TLP-ste) and Bacillus thermoproteolyticus (thermolysine). Boiling-resistant and calcium independent or dependent metallo-endopeptidases can be applied in several industrial processes, for instance in the preparation of the artificial sweetener aspartame, but also in the leather industry, in breweries and in the production of protein hydrolysates.

Brief Summary Text - BSTX (1):

The present invention relates to metallo-endopeptidases, sometimes also called neutral proteases, that are produced, processed and secreted by e.g. members of the bacterial genus Bacillus. The present invention provides genes encoding variants of metallo-enopeptidases that have been engineered to be resistant to prolonged boiling while maintaining their enzymatic performance at much lower temperatures. In addition, thermal stability of the metallo-endopeptidases is highly dependent on calcium at concentrations in the mM range. The invention thus further provides active metallo-endopeptidase **variants whose stability** depending on calcium concentration can be changed so as to provide metallo-endopeptidases that are calcium dependent or independent. The invention also provides genes that encode boiling-resistant metallo-endopeptidases whose stability depending on calcium concentration can be changed. The invention also provides vectors and cells comprising these genes and proteases produced through these genes, vectors and/or cells.

Brief Summary Text - BSTX (5):

The invention also provides **calcium-dependent and--independent variants** of thermolysine-like proteases. Another embodiment of the invention is a recombinant DNA molecule comprising a least a functional part of the sequence of FIG. 7B (SEQUENCE ID No. 2) or FIG. 7A (SEQUENCE ID No. 1) coding for a polypeptide having metallo-endopeptidase activity wherein at least one codon is mutated to code for an amino-acid providing the resulting gene product (polypeptide) with a reduced capacity to bind with calcium.

Brief Summary Text - BSTX (6):

For example, (i) referring to FIG. 7B (SEQUENCE ID No. 2), the Ca.sub.3 binding site of TLP-ste was deteriorated by mutating one of the main ligands (Asp57), but substitutions T63F or A69P leave the calcium-dependent stability intact, and (ii) referring to FIG. 7A (SEQUENCE ID No. 1), the Ca.sub.3 binding site of TLP-ste was deteriorated by mutating one of the main ligands (Asp60) but substitutions T66F or A72P leave the calcium-dependent stability intact. Subsequently, the loss in stability is compensated for by introducing stabilising mutations in the direct environment of the Ca.sub.3 site. The results confirm the importance of the Ca.sub.3 site for stability and they show the feasibility of engineering various grades of **calcium-dependency in otherwise stable variants**.

Brief Summary Text - BSTX (18):

The half-life of the 8-fold mutant at 100.degree. C. was more than 1000 times that of the wild-type and the temperature for optimum activity was raised by 21.degree. C. The specific activities at 37.degree. C. were identical for the wild-type TLP-ste and the 8-fold mutant (Table 1). In contrast to the wild-type, the 8-fold mutant was active and stable in the presence of high concentrations of denaturing agents (Table 2). The cleavage specificity at both moderate and high temperatures was largely unaffected by the stabilizing mutations (Table 1 and FIG. 3). In summary, the enzymatic properties of the constructed **variant resemble those of the wild-type, but its stability** resembles that of extremozymes or thermozymes produced by organisms that are capable of surviving in extreme environments such as Archaea and Eubacterial extremophiles.

Brief Summary Text - BSTX (19):

As an example of the enzymatic activity of the 8-fold **mutant** we tested the hydrolysis of protease resistant **.alpha.-amylase from Bacillus** licheniformis by the 8-fold **mutant**. B. licheniformis **.alpha.-amylase** (1 mg/ml) in 50 mM MOPS, pH 7.0, 5 mM CaCl.sub.2, 0.01% Triton X-100 was incubated with purified TLP-ste (1 .mu.g/ml), the 8-fold **mutant** or without protease for 60 minutes at the temperature indicated. The reaction volume was 500 .mu.L. After incubation the samples were cooled on ice, which resulted in aggregation of the substrate in the samples that had been incubated at 100.degree. C. Precipitates (only observed in the 100.degree. C. samples) were collected by centrifugation and redissolved in 500 .mu.L 6 M Urea. Both supernatants and redissolved precipitates were subjected to standard SDS-PAGE, including pre-treatment with sample loading buffer (5 minutes at 100.degree. C.). The samples were

identical in size (20 .mu.L supernatant and 20 .mu.L dissolved precipitate). Gels were stained with Coomassie-brilliant blue. No significant degradation of **.alpha.-amylase** occurred at temperatures of 80.degree. C. and lower, irrespective of the enzyme used. In case the samples were incubated at 100.degree. C. without added protease or with TLP-ste the aggregate formed after cooling contained mature **.alpha.-amylase,** indicating that no hydrolysis had occurred. The B. licheniformis **.alpha.-amylase** that was incubated with the 8-fold **mutant** at 100.degree. C. was completely hydrolysed and no aggregate was formed.

Brief Summary Text - BSTX (22):

With regard to calcium binding (referring to FIGS. 7A and 7B and SEQUENCE ID No. 2), it was found that from a structural point of view Asp57 seemed more important for calcium binding than Asp59 because both Ods of Asp57 interact with the calcium versus only one Od of Asp59 (FIG. 4). Asp57 was replaced by Ser because in the less thermostable TLPs residue 57 is a serine. From a visual inspection of the three dimensional environment of residue 57 it was concluded that the D57S mutation would not have additional negative effects such as disturbance of the local hydrogen bonding network or the introduction of clashes. To compensate the expected destabilising effect of this mutation, the combined T63F-A69P mutation was chosen. The stabilising mutations had been identified in previous site-directed mutagenesis studies of differences between naturally occurring TLPs (Van den Burg, B., Enequist, H. G., Van der Haar, M. E., Eijsink, V. G. H., Stulp, B. K. and Venema, G. (1991) J. Bacteriol. 173, 4107-4115) and in studies concerning the design of stabilising Xxx->Pro mutations in TLP-ste (Hardy, F., Vriend, G., Veltman, O. R., van der Vinne, B., Venema, G. and Eijsink, V. G. H. (1993) FEBS Lett. 317, 89-92). The mutations are located in the direct environment of Ca.sub.3 and the double mutation had previously been shown to drastically stabilise TLP-ste. Characteristics of the various **mutants, including the dependence of stability** on calcium concentration are presented in Tables 5 and 6 and in FIGS. 5 and 6. As shown in Table 5, the wild-type and mutant enzymes were similar with respect to their activity towards FaGLa.

Brief Summary Text - BSTX (24):

The **stability of TLP-ste and the T63F-A69P mutant** (referring to FIGS. 7A and 7B and SEQUENCE ID No. 2) (which both have the Ca.sub.3 site intact) depended strongly on the calcium concentration (FIG. 5, Tables 4, 5). Introduction of the D57S mutation reduced this calcium dependence. Consequently, the destabilising effect of the D57S mutant became smaller with decreasing calcium concentration; at the lowest calcium concentration tested, the wild-type enzyme was even slightly stabilised by the D57S mutation). The stability versus calcium concentration curves of TLP-ste and T63F-A69P (FIG. 5) can be superimposed remarkably well. The same is true for the D57S and the D57S-T63F-A69P, strongly suggesting that the observed effects on the calcium stability are indeed caused by the disturbance of the Ca.sub.3 site by the D57S mutation.

Brief Summary Text - BSTX (25):

The D57S-T63F-A69P mutant (referring to FIGS. 7A and 7B and SEQUENCE ID No.

2) represents a TLP-ste **variant wh se stability** is largely independent of the calcium concentration and which, at lower calcium concentrations, is considerably more stable than the wild-type enzyme (Table 5). Combining known stabilising mutations in the Ca.sub.3 region has resulted in extremely stable TLP-ste variants. Therefore, it is likely that mutants can be designed that are even less dependent on calcium than the ones described here and that are more stable. Also, and on the other hand, the knowledge now obtained about the calcium-binding site can be used to design **variants that are still calcium-dependent** while at the same time having obtained a much higher resistance to elevated temperatures or that are even resistant to boiling. Such enzymes can be used in reactions that require prolonged boiling but that can be stopped by changing the calcium concentration in the reaction mixture, by for example adding calcium or chelating agents that capture calcium, depending on the needs of the protease used. Engineering calcium-independence does not necessarily need to be based on detonating the Ca.sub.3 site. Instead, it could be based on adding mutations that stabilise the local structure, regardless of the presence of a calcium ion. For example, preliminary analyses of a mutant in which the (intact) calcium binding site is covalently cross linked with the N-terminal .beta.-hairpin showed that the **stability of this mutant is also less calcium-dependent**.

Detailed Description Paragraph Table - DETL (4):

TABLE 4 Activity and **stability of TLP-ste variants.** FaGLa, t1/2 at t1/2 at k.sub.cat /K.sub.m 5 mM 0.2 mM Half-life .times. 10.sup.-3 CaCl.sub.2 CaCl.sub.2 ratio Variant (M.sup.-1 .multidot. S.sup.-1) (min) (min) 5 mM/0.2 mM TLP-ste 34 31 2.6 12 D57S 22 5.8 3.8 1.5 T63F-A69P 21 990 52 19 D57S-T63F-A 28 64 42 1.5 69P

US-PAT-NO: 6482622

DOCUMENT-IDENTIFIER: US 6482622 B1

TITLE: Amylolytic enzyme variants

DATE-ISSUED: November 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cherry; Joel Robert	Davis	CA	N/A	N/A
Svendsen; Allan	Birkerod	N/A	N/A	DK
Andersen; Carsten Denmark	Vaerloese	N/A	N/A	DK
Beier; Lars	Lyngby	N/A	N/A	DK
Frandsen; Torben Peter	Frederiksberg C	N/A	N/A	DK
Schafer; Thomas	Farum	N/A	N/A	DK

APPL-NO: 09/ 645707

DATE FILED: August 24, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK/99/00087 filed on Feb. 26, 1999, and claims priority under 35 U.S.C. 119 of Danish application nos. PA 1998 00269 and PA 1998 00273, both filed on Feb. 27, 1998, and U.S. provisional application Nos. 60/077,509 and 60/077,795, filed on Mar. 11, 1998 and Mar. 12, 1998, respectively, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1998 00273	February 27, 1998
DK	1998 00269	February 27, 1998

US-CL-CURRENT: 435/193, 435/183 , 435/200 , 435/201 , 435/202

ABSTRACT:

The inventors have discovered some striking, and not previously predicted structural similarities and differences between the structure of Novamyl and the reported structures of CGTases, and based on this they have constructed variants of maltogenic alpha-amylase having CGTase activity and variants of CGTase having maltogenic alpha-amylase activity. Further, on the basis of sequence homology between Novamyl.RTM. and CGTases, the inventors have constructed hybrid enzymes with one or more improvements to specific properties of the parent enzymes, using recombinant DNA methodology.

24 Claims, 5 Drawing figures

Exemplary Claim Number: 6

Number of Drawing Sheets: 5

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Detailed Description Text - DETX (79):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a maltogenic **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (95):
To screen for **variants with increased stability,** the filter with bound maltogenic alpha-amylase variants can be pretreated prior to the detection step described above to inactivate **variants that do not have improved stability** relative to the parent CGTase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detailed Description Text - DETX (96):
To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent CGTase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Red labeled

amylopectin and incubated until activity is detectable. Similarly, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent CGTase is inactivated, thereby allowing detection of only those **variants with increased stability** at the pH in question. To screen for **variants with increased calcium-dependent** stability calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent CGTase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

Detailed Description Text - DETX (150):

In this example, the unique active site loop was used to select hybrid enzymes with maltogenic **alpha-amylase** activity from a library of random recombinants. In this method, Novamyl and the cyclic maltodextrin glucosyl transferase (CGTase) from **Bacillus** circulans, were randomly recombined by the DNA shuffling method of Crameri A, et al., op.cit. Those resulting **mutants** containing the Novamyl loop were selected using PCR as described above in Example 2.

US-PAT-NO: 6479451

DOCUMENT-IDENTIFIER: US 6479451 B2

TITLE: Laundry detergent compositions comprising hydrophobically modified polyamines and nonionic surfactants

DATE-ISSUED: November 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Kenneth Nathan	Cincinnati	OH	N/A	N/A

APPL-NO: 09/ 790042

DATE FILED: February 21, 2001

PARENT-CASE:

CROSS-REFERENCE

This Application claims the benefit of U.S. Provisional Application No. 60/184,250, filed on Feb. 23, 2000.

US-CL-CURRENT: 510/303, 510/309 , 510/310 , 510/311 , 510/312 , 510/336 , 510/340 , 510/341 , 510/350 , 510/351 , 510/356 , 510/360 , 510/499

ABSTRACT:

The present invention relates to laundry detergent compositions comprising one or more hrdrophobically modified polyamines and nonionic surfactants which provide enhanced hydrophilic soil, inter alia, clay, removal benefits. The present invention also relates to methods for removing hydrophilic soil from wearing apparel.

18 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (99):

A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl

hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Brief Summary Text - BSTX (101):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Brief Summary Text - BSTX (126):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B.

subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6472359

DOCUMENT-IDENTIFIER: US 6472359 B1

TITLE: Laundry detergent compositions comprising zwitterionic polyamines and xyloglucanase

DATE-ISSUED: October 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ghosh; Chanchal Kumar	West Chester	OH	N/A	N/A

APPL-NO: 09/ 790044

DATE FILED: February 21, 2001

PARENT-CASE:

CROSS REFERENCE

This Application claims the benefit of U.S. Provisional Application No. 60/184,367, filed on Feb. 23, 2000

US-CL-CURRENT: 510/321, 510/338 , 510/340 , 510/351 , 510/356 , 510/357 , 510/360 , 510/373 , 510/504 , 510/530 , 562/107

ABSTRACT:

The present invention solves the problem of soil and dirt becoming entrained in cellulosic material loosened and removed from fabric during washing wherein said dirt and soil is entrapped by the cellulosic material and re-deposited onto the fabric surface. The compositions of the present invention comprise: a) from about 0.01% by weight, of a zwitterionic polyamine which comprises a polyamine backbone, said backbone comprising two or more amino units wherein at least one of said amino units is quaternized and wherein at least one amino unit is substituted by one or more moieties capable of having an anionic charge wherein further the number of amino unit substitutions which comprise said anionic moiety is less than or equal to the number of quaternized backbone amino units; b) from about 0.00005% by weight, of a xyloglucanase enzyme; c) from about 0.5% to about 50% by weight, of a surfactant system comprising: i) from about 10% to about 99% by weight, of said surfactant system, of a nonionic surfactant; ii) from about 1% to about 90% by weight, of said surfactant system, of an anionic surfactant; iii) optionally, from 1% to about 50% by weight, of said surfactant system, of a detersive surfactant selected from the group consisting of cationic surfactants, zwitterionic surfactants, ampholytic surfactants, and mixtures thereof; and d) the balance carriers and adjunct ingredients.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (47):

A preferred protease enzyme for use in the present invention is a variant of Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detailed Description Text - DETX (49):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (74):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using

site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17, 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6440922

DOCUMENT-IDENTIFIER: US 6440922 B1

TITLE: Detergent composition comprising zeolite and amylase enzyme

DATE-ISSUED: August 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Garnett; Carolyn Jayne	Brussels	N/A	N/A	BE
Clare; Jonathan Richard	Newcastle upon Tyne	N/A	N/A	GB
Wauben; Johan Juliaan Serafin	Lint	N/A	N/A	BE

APPL-NO: 08/ 860941

DATE FILED: July 14, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9500738	January 14, 1995

PCT-DATA:
APPL-NO: PCT/US96/00281
DATE-FILED: January 5, 1996
PUB-NO: WO96/21717
PUB-DATE: Jul 18, 1996
371-DATE: Jul 14, 1997
102(E)-DATE:Jul 14, 1997

US-CL-CURRENT: 510/392, 510/309 , 510/312 , 510/313 , 510/320 , 510/321 , 510/334 , 510/507 , 510/530 , 510/532

ABSTRACT:

A detergent composition comprises a zeolite builder having a particle size, expressed as a d.sub.50 value, of less than 1.0 micrometers, an amylase enzyme, and an alkoxylated nonionic surfactant having a hydrophilic lipophilic balance value of less than 9.5 selected from the group consisting of alkoxylated adducts of fatty alcohols containing an average of less than 5 alkylene oxide groups per molecule. The detergent composition has been found to reduce white residue formation on fabrics washed with detergent containing small particle size zeolite.

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (39):

Highly preferred amylases with enhanced oxidative stability are derived using site-directed mutagenesis from one or more of the Baccillus amylases, especialy the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Preferred amylases of this type are described in WO 94/02597 A, and comprise a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **.alpha.-amylase,** sold under the tradename Termamyl, or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B.subtilis, or B.stearothermophilus.

Brief Summary Text - BSTX (89):

In the detergent compositions, the abbreviated component identifications have the following meanings: LAS: C.sub.11 -C.sub.13 linear alkyl benzene sulfonate 45AS: Branched sodium alkyl sulfate surfactant containing C.sub.14 -C.sub.15 alkyl chains 246AS: Sodium alkyl sulfate surfactant containing a alkyl chain length weight distribution of 15% C.sub.12 alkyl chains, 45% C.sub.14 alkyl chains, 35% C.sub.16 alkyl chains, 5% C.sub.18 alkyl chains TAS: Sodium alkyl sulfate surfactant containing predominantly C.sub.16-C.sub.18 alkyl chains derived from tallow oil. 24AE3S: C.sub.12 -C.sub.14 alkyl ethoxysulfate containing an average of three ethoxy groups per mole 35E3: A C.sub.13-15 primary alcohol condensed with an average of 3 moles of ethylene oxide 25E3: A C.sub.12 -C.sub.15 primary alcohol condensed with an average of 3 moles of ethylene oxide 24EY: A C.sub.12-14 linear primary alcohol condensed with an average of Y moles of ethylene oxide Citrate: Sodium citrate Carbonate: Anhydrous sodium carbonate Perborate: Sodium perborate tetrahydrate Percarbonate: Sodium percarbonate TAED: Tetra acetyl ethylene diamine Silicate: Amorphous Sodium Silicate (SiO.sub.2 :Na.sub.2 O ratio normally follows) CMC: Carboxymethylcellulose Suds: 25% paraffin wax Mpt 50.degree. C., 17% Suppressor hydrophobic silica, 58% paraffin oil Zeolite MAP: Hydrated sodium aluminosilicate zeolite MAP having a silicon to aluminium ratio of 1.07 having a particle size, expressed as a d.sub.50 value, of 0.5 micrometres Zeolite A: Hydrated sodium aluminosilicate zeolite A having a particle size, expressed as a d.sub.50 value, of 0.6 micrometres MA/AA: Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000. Amylase: Amylolytic enzyme sold under the tradename Termamyl 60T by Novo Industries A/S (60 KNU/gram enzyme activity) BSA: Amylolytic enzyme--M197T **variant, having enhanced oxidative stability** (60 KNU/gram enzyme activity) Protease: Proteolytic enzyme sold by Novo Industries A/S under the tradename Savinase of activity 4.0 KNPU/gram. Lipase: Lipolytic enzyme sold by Novo Industries A/S under the tradename lipolase of activity 100,000 LU/gram

US-PAT-NO: 6440716

DOCUMENT-IDENTIFIER: US 6440716 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen	N/A	N/A	DK

APPL-NO: 09/ 636252

DATE FILED: August 10, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/327,563 filed Jun. 8, 1999, which is a continuation of U.S. application Ser. No. 08/683,838 filed Jul. 18, 1996, now U.S. Pat. No. 6,022,724 which is a continuation-in-part of U.S. application Ser. No. 08/600,908 filed Feb. 13, 1996, now U.S. Pat. No. 5,989,169 which is a 371 national of PCT/DK96/00057 filed Feb. 5, 1996, and claims priority under 35 U.S.C. 119 of Danish application .0128/95 filed Feb. 3, 1995, Ser. No. 1192/95 filed Oct. 23, 1995 and Ser. No. 1256/95 filed Nov. 10, 1995, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0128/95	February 3, 1995
DK	1192/95	October 23, 1995
DK	1256/95	November 10, 1995

US-CL-CURRENT: 435/202

ABSTRACT:

The present invention relates to a method of constructing a variant of a parent Termamyl-like .alpha.-amylase, which variant has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprises i) analysing the structure of the parent Termamyl-like .alpha.-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like .alpha.-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent Termamyl-like .alpha.-amylase (as evaluated

on the basis of structural or functional considerations), ii) constructing a Termamyl-like .alpha.-amylase variant, which as compared to the parent Termamyl-like .alpha.-amylase, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and, optionally, iii) testing the resulting Termamyl-like .alpha.-amylase variant with respect to the property in question.

13 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Detailed Description Text - DETX (213):
.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly indeionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID NO: 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Detailed Description Text - DETX (220):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

US-PAT-NO: 6436888

DOCUMENT-IDENTIFIER: US 6436888 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Borchert; Torben Vedel	Jyllinge	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK

APPL-NO: 09/ 672459

DATE FILED: September 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/182,859 filed Oct. 29, 1998, now U.S. Pat. No. 6,143,708, which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0515/96	April 30, 1996
DK	0712/96	June 28, 1996
DK	0775/96	July 11, 1996
DK	1263/96	November 8, 1996

US-CL-CURRENT: 510/226, 435/202 , 435/252.3 , 435/320.1 , 510/326 , 510/392 , 536/23.2 , 536/23.7

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

16 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Among more recent disclosures relating to **.alpha.-amylases**, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 4 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 2 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (101):

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which **variant exhibits increased stability** at low pH and at low calcium concentration relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

Brief Summary Text - BSTX (128):

.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Brief Summary Text - BSTX (135):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (14):

The random mutagenesis may be carried out by the following steps: 1. Select regions of interest for modification in the parent enzyme 2. Decide on mutation sites and nonmutated sites in the selected region 3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed 4. Select structurally reasonable mutations. 5. Adjust the residues selected by step 3 with regard to step 4. 6. Analyze by use of a suitable dope algoritm the nucleotide distribution. 7. If necessary, adjust the wanted residues to genetic code realism (e.g. taking into account constraints resulting from the genetic code (e.g. in order to avoid introduction of stop codons))(the skilled person will be aware that some codon combinations cannot be used in practice and will need to be adapted) 8. Make primers 9. Perform random mutagenesis by use of the primers 10. Select resulting .alpha.-amylase variants by screening for the desired improved properties.

Detailed Description Text - DETX (58):

Construction, by localized random, doped mutagenesis, of Termamyl-like .alpha.-amylase **variants having an improved stability** at low pH and a reduced dependency on calcium ions for stability compared to the parent enzyme

Detailed Description Text - DETX (59):

.alpha.-amylases are of great importance for the industrial starch liquefaction process. The variant of the thermostable B. licheniformis .alpha.-amylase consisting of amino acids 1-33 of the B. amyloliquefaciens amylase (SEQ ID NO 4) fused to amino acids 36-483 of the B. licheniformis amylase (SEQ ID NO 2) and further comprising the following mutations: Y156, T181, F190, V209 and S264 (the construction of this **variant is described in Example 1) has a very satisfact ry stability** at low pH and low calcium concentrations. In an attempt to further improve the stability at low pH and

low calcium concentration of said .alpha.-amylase variant random mutagenesis in preselected regions wase performed.

Detailed Description Text - DETX (85):

The mutations indicated in bold were introduced by the 35 random mutagenesis method. The **stability data for these variants** appear from Table 11 in Example 3.

Detailed Description Text - DETX (101):

This example summarises the **stability results of variants** characterised by a fluorimetric assay at 70.degree. C. under two different conditions, (1) pH 4.5 and 1 mM CaCl.sub.2 and (2) pH 6.2 and 10 .mu.M CaCl.sub.2.

Claims Text - CLTX (5):

5. A **variant** as defined in claim 1, wherein the parent Termamyl-like **.alpha.-amylase** is selected from the group consisting of: the B. licheniformis **.alpha.-amylase** having the sequence shown in SEQ ID No. 2, the B. amyloliquefaciens **.alpha.-amylase** having the sequence shown in SEQ ID No. 4, the B. stearothennophilus **.alpha.-amylase** having the sequence shown in SEQ ID No. 6, the **Bacillus** strain NCIB 12512 **.alpha.-amylase** having the sequence shown in FIG. 1 and 2, the **Bacillus** strain NCIB 12513 **.alpha.-amylase** having the sequence shown in FIG. 2, and the **Bacillus** sp. #707 **.alpha.-amylase** having the sequence shown in FIG. 2.

US-PAT-NO: 6410295

DOCUMENT-IDENTIFIER: US 6410295 B1

TITLE: Alpha-amylase variants

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Andersen; Carsten	Vaerl.o slashed.se	N/A	N/A	DK
J.o slashed.rgensen; Christel K .o slashed.	benhavn .O	N/A	N/A	DK
Thea	slashed.	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Kjaerulff; S.o slashed.ren	Vanl.o slashed.se			

APPL-NO: 09/ 537168

DATE FILED: March 29, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish application PA 1999 00437 filed Mar. 30, 1999, and of U.S. Provisional application No. 60/127,427 filed Apr. 1, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	00437/99	March 30, 1999

US-CL-CURRENT: 435/202, 435/183 , 435/200 , 435/252.3 , 435/320.1 , 435/440

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like alpha-amylase, which variant exhibits altered properties, in particular reduced capability of cleaving a substrate close to the branching point, and improved substrate specificity and/or improved specific activity relative to the parent alpha-amylase.

33 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (6):

Among recent disclosure relating to **alpha-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **alpha-amylase,** reffered to as BA2, which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **alpha-amylase** comprising the amino acid sequence shown in SEQ ID NO: 6 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis **alpha-amylase** comprising the amino acid sequence shown in SEQ ID NO: 4 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus alpha-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **alpha-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **alpha-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **alpha-amylase, variants** of the parent Termamyl-like **alpha-amylase** which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (224):

(c) screening for host cells expressing an alpha-amylase **variant which has an altered property (i.e., thermal stability**) relative to the parent alpha-amylase.

Brief Summary Text - BSTX (235):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **alpha-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **alpha-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **alpha-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **alpha-amylase,** A. niger acid stable **alpha-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

US-PAT-NO: 6361989

DOCUMENT-IDENTIFIER: US 6361989 B1

TITLE: .alpha.-amylase and .alpha.-amylase variants

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerod	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen	N/A	N/A	DK
Bisgard-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK
Outtrup; Helle	Ballerup	N/A	N/A	DK
Nielsen; Bjarne Ronfeldt	Virum	N/A	N/A	DK
Nielsen; Vibeke Skovgaard	Bagsv.ae butted.rd	N/A	N/A	DK
Hedegaard; Lisbeth	Skodsborg	N/A	N/A	DK

APPL-NO: 09/ 290734

DATE FILED: April 13, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Continuation-in-Part of U.S. application Ser. No. 09/170,670 filed on Oct. 13, 1998 now U.S. Pat. No. 6,187,576 issued on Feb. 13, 2001 which claims priority of Provisional application No. 60/063,306 filed Oct. 28, 1997 and Danish application no. 1172/97 filed on Oct. 13, 1997, and further claims priority of Danish application no. PA 1999 00439 filed Mar. 31, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1172/97	October 13, 1997
DK	1999 00439	March 31, 1999

US-CL-CURRENT: 435/202, 435/183 , 435/200

ABSTRACT:

The invention relates to a novel Termamyl-like .alpha.-amylase, and Termamyl-like .alpha.-amylases comprising mutations in two, three, four, five or six regions/positions. The variants have increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the

invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent).

5 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Brief Summary Text - BSTX (6):

Among more recent disclosures relating to **.alpha.-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Detailed Description Text - DETX (230):

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent .alpha.-amylase, e.g. wherein the **variant exhibits altered or increased thermal stability** relative to the parent, the method comprising: (a) subjecting a DNA sequence encoding the parent .alpha.-amylase to random mutagenesis, (b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and (c) screening for host cells expressing an .alpha.-amylase **variant which has an altered property (i.e. thermal stability**) relative to the parent .alpha.-amylase.

Detailed Description Text - DETX (250):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA

sequence encoding an .alpha.-amylase variant of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the Bacillus licheniformis .alpha.-amylase gene (amyL), the promoters of the Bacillus stearothermophilus maltogenic amylase gene (amyM), the promoters of the Bacillus amyloliquefaciens .alpha.-amylase (amyQ), the promoters of the Bacillus subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral .alpha.-amylase, A. niger acid stable .alpha.-amylase, A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (284):

Amylases: Suitable amylases (.alpha.- and/or .beta.-) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, .alpha.-amylases obtained from Bacillus, e.g., a special strain of B. licheniformis, described in more detail in GB 1,296,839.

Detailed Description Text - DETX (327):

Enzymes: SP690: .alpha.-amylase shown in SEQ ID NO: 1 SP722: .alpha.-amylase shown in SEQ ID NO: 2 Termamyl.RTM.: .alpha.-amylase from Bacillus licheniformis shown in SEQ ID NO: 4. AA560: .alpha.-amylase of the invention shown in SEQ ID NO: 24 encoded by the DNA sequence shown in SEQ ID NO: 23. AA360: .alpha.-amylase shown in SEQ ID NO: 26 being identical to the AA560 .alpha.-amylase encoded by the DNA sequence shown in SEQ ID NO: 25. BSG alpha-amylase: B. stearothermophilus alpha-amylase depicted in SEQ ID NO: 3. TVB146 alpha-amylase variant: B. stearothermophilus alpha-amylase variant depicted in SEQ ID NO: 3 with the following mutations: with the deletion in positions I181-G182+N193F. LE174 hybrid alpha-amylase variant: LE174 is a hybrid Termamyl-like alpha-amylase being identical to the Termamyl sequence, i.e., the Bacillus licheniformis .alpha.-amylase shown in SEQ ID NO: 4, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the Bacillus amyloliquefaciens alpha-amylase shown in SEQ ID NO: 5, which further have the following mutations: H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 4). LE174 was constructed by SOE-PCR (Higuchi et al. 1988, Nucleic Acids Research 16:7351).

US-PAT-NO: 6352851

DOCUMENT-IDENTIFIER: US 6352851 B1

TITLE: Glucoamylase variants

DATE-ISSUED: March 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; Bjarne Roenfeldt	Virum	N/A	N/A	DK
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Pedersen; Henrik	Odense	N/A	N/A	DK
Vind; Jesper	Lyngby	N/A	N/A	DK
Hendriksen; Hanne Vang	Holte	N/A	N/A	DK
Frandsen; Torben Peter	Frederiksberg	N/A	N/A	DK

APPL-NO: 09/ 351814

DATE FILED: July 12, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish application Nos. PA 1998 00937 and PA 1998 01667 filed Jul. 15, 1998 and Dec. 17, 1998 and U.S. provisional application Nos. 60/093,528 and 60/115,545 filed Jul. 21, 1998 and Jan. 12, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1998 00937	July 15, 1998
DK	1998 01667	December 17, 1998

US-CL-CURRENT: 435/205, 435/183 , 435/200 , 435/202 , 435/203

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits improved thermal stability and/or increased specific activity using saccharide substrates.

6 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Detailed Description Text - DETX (259):
In a second aspect the invention relates to a **variant of a parent glucoamylase with improved thermal stability,** in particular in the range from 40-80.degree. C., preferably 60-80.degree. C., and preferably at pH 4-5, said variant comprising one or more mutation(s) in the following position(s) or region(s) in the amino acid sequence shown in NO: 2:

Detailed Description Text - DETX (539):
In a preferred embodiment the **variant of the invention has improved thermal stability** and/or increased specific activity, preferably within the temperature interval from about 60-80.degree. C., preferably 63-75.degree. C., preferably at a pH of 4-5, in particular 4.2-4.7, using maltodextrin as the substrate.

Detailed Description Text - DETX (554):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent glucoamylase, wherein the **variant exhibits increased thermal stability** relative to the parent, the method comprising:

Detailed Description Text - DETX (557):
(c) screening for host cells expressing a glucoamylase **variant which has an altered property (i.e. thermal stability**) relative to the parent glucoamylase.

Detailed Description Text - DETX (579):
Examples of suitable promoters for directing the transcription of the DNA sequence encoding a glucoamylase **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, the TPI (triose phosphate isomerase) promoter from S. cerevisiae (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (605):
Further, by improving the thermal stability the T.sub.1/2 (half-time, as defined in the "Materials and Methods" section) is improved. As the thermal

stability f the gluc amylas variants of the invention is improved a minor amount of glucoamylase need to be added to replace the glucoamylase being inactivated during the saccharification process. More glucoamylase is maintained active during saccharification process according to the present invention. Furthermore, the risk of microbial contamination is also reduced when carrying the saccharification process at temperature above 63.degree. C.

Detailed Description Text - DETX (664):

The thermal **stability of variants** is determined as T.sub.1/2 using the following method: 950 microliter 50 mM sodium acetate buffer (pH 4.3) (NaOAc) is incubated for 5 minutes at 68.degree. C. or 70.degree. C. 50 microliter enzyme in buffer (4 AGU/ml) is added. 2.times.40 microliter samples are taken at 0, 5, 10, 20, 30 and 40 minutes and chilled on ice. The activity (AGU/ml) measured before incubation (0 minutes) is used as reference (100%). The decline in stability (in percent) is calculated as a function of the incubation time. The % residual glucoamylase activity is determined at different times. T.sub.1/2 is the period of time until which the % relative activity is decreased to 50%. T.sub.T1/2 (half-life) (Method II) The T.sub.1/2 is measured by incubating the enzyme (ca 0.2 AGU/ml) in question in 30% glucose, 50 mM Sodium acetate at pH 4.5 at the temperature in question (e.g., 70.degree. C.). Samples are withdrawn at set time intervals and chilled on ice and residual enzyme activity measured by the pNPG method (as described below).

Detailed Description Text - DETX (690):

3. Decide on which kind of mutations should be carried out, e.g., with respect to the desired **stability and/or performance of the variant** to be constructed,

US-PAT-NO: 6350599

DOCUMENT-IDENTIFIER: US 6350599 B1

TITLE: Pullulanase variants and methods for preparing such variants with predetermined properties

DATE-ISSUED: February 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerod	N/A	N/A	DK

APPL-NO: 09/ 514599

DATE FILED: February 28, 2000

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	PA20000045	January 20, 2000

US-CL-CURRENT: 435/183, 530/300 , 530/350 , 536/23.1

ABSTRACT:

The inventors have modified the amino acid sequence of a pullulanase to obtain variants with improved properties, based on the three-dimensional structure of the pullulanase Promozyme.RTM.. The variants have altered physicochemical properties, e.g. an altered pH optimum, improved thermostability, altered substrate specificity, increased specific activity or an altered cleavage pattern.

8 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

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Detailed Description Text - DETX (91):
Pullulanase **Variants with Altered Stability**

Detailed Description Text - DETX (92):
A **variant with improved stability** (typically increased thermostability) may be obtained by substitution with proline, substitution of histidine with another amino acid, introduction of a disulfide bond, removal of a deamidation

site, altering a hydrogen bond contact, filling in an internal structural cavity with one or more amino acids with bulkier side groups, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

Detailed Description Text - DETX (122):
Furthermore, it is envisaged from the structure that deletion of certain amino acid residues will confer increased **stability, such as increased thermostability, to the thus produced variant**. Variants, which are believed to be of particular importance, comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detailed Description Text - DETX (124):
Other deletions which are expected to confer increased **stability, such as increased thermostability, to the pullulanase variant** comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detailed Description Text - DETX (126):
Furthermore, the following deletions are expected to confer increased **stability, such as increased thermostability, to the pullulanase variant** comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 1:

Detailed Description Text - DETX (129):
For example, it is envisaged that deletion of the below amino acid residues will confer increased **stability, such as increased thermostability, to the thus produced variant** of the pullulanase from Bacillus deramificans (SEQ ID NO: 3):

Detailed Description Text - DETX (131):
Other deletions which are expected to confer increased **stability, such as increased thermostability, to the pullulanase variant** comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detailed Description Text - DETX (133):
Furthermore, the following deletions are expected to confer increased **stability, such as increased thermostability, to the pullulanase variant** comprises a deletion of amino acid residues corresponding to the following residues of the amino acid sequence set forth in SEQ ID NO: 3:

Detailed Description Text - DETX (148):
h) selecting a **variant having increased stability** and/or an altered temperature dependent activity profile as compared to the parent pullulanase.

Detailed Description Text - DETX (161):
A **variant with impr ved stability** (typically improved thermostability) as compared to the parent pullulanase may be obtained by introducing new interdomain and intradomain contacts, such as establishing inter- or intradomain disulfide bridges.

Detailed Description Text - DETX (168):
f) testing the **stability of said variant**; and

Detailed Description Text - DETX (170):
h) selecting a **variant having increased stability** as compared to the parent pullulanase.

Detailed Description Text - DETX (178):
A **variant with improved stability** (typically improved thermostability) as compared to the parent pullulanase may be obtained by changing the surface charge distribution of the pullulanase. For example, when the pH is lowered to about 5 or below histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the pullulanase one may avoid such unfavorable electrostatic interactions which in turn leads to a higher stability of the pullulanase.

Detailed Description Text - DETX (185):
f) testing the **stability of said variant**; and

Detailed Description Text - DETX (187):
h) selecting a **variant having increased stability** as compared to the parent pullulanase.

Detailed Description Text - DETX (207):
Variants with improved stability, in particular variants with improved thermostability, can be obtained by improving existing or introducing new interdomain or intradomain contacts. Such improved stability can be achieved by the modifications listed below.

Detailed Description Text - DETX (208):
Thus, one preferred embodiment of the invention relates to a **variant of a parent pullulanase which has an improved stability** and one or more salt bridges as compared to the parent pullulanase, wherein said variant comprises a modifications, e.g. a substitution, in a position corresponding to at least one of the following sets of positions in SEQ ID NO: 1:

Detailed Description Text - DETX (408):

3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or perf rmance of the variant** to be constructed

Detailed Description Text - DETX (421):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a pullulanase **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (437):

To screen for **variants with increased stability, the filter with bound pullulanase variants** can be pretreated prior to the detection step described above to inactivate **variants that do not have improved stability** relative to the parent pullulanase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from pH 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Detailed Description Text - DETX (438):

To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent pullulanase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Blue labeled pullulan and incubated until activity is detectable. As will be understood, thermostability and increased isoamylase activity may be tested simultaneously by using a detection plate containing immobilized Cibacron Red labeled

amylopectin and incubate until activity is detectable. Moreover, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent pullulanase is inactivated, thereby allowing detection of only those **variants with increased stability** at the pH in question. To screen for **variants with increased calcium-dependent** stability, calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent pullulanase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

US-PAT-NO: 6329186

DOCUMENT-IDENTIFIER: US 6329186 B1

TITLE: Glucoamylases with N-terminal extensions

DATE-ISSUED: December 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; R.o slashed.nfeldt	Virum	N/A	N/A	DK
Bjarne	Birker.o slashed.d	N/A	N/A	DK
Svendsen; Allan	Hellerup	N/A	N/A	DK
Bojsen; Kirsten	Lyngby	N/A	N/A	DK
Vind; Jesper	Bagsv.oe butted.rd	N/A	N/A	DK
Pedersen; Henrik				

APPL-NO: 09/ 455679

DATE FILED: December 7, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish applications PA 1998 01616 filed Dec. 7, 1998 and PA 1999 00409 filed Mar. 24, 1999, and of U.S. Provisional applications No. 60/111,674 filed Dec. 10, 1998 and No. 60/126,740 filed Mar. 29, 1999, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1998 01616	December 7, 1998
DK	1999 00409	March 24, 1999

US-CL-CURRENT: 435/205, 435/183 , 435/200

ABSTRACT:

The invention relates to a variant of a parent fungal glucoamylase, which exhibits improved thermal stability.

28 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (30):

Stability: The peptide extension should preferably be chosen so as to provide a glucoamylase **variant with an acceptable stability** (e.g., structural stability and/or expression stability) or so as to not significantly reduce the structural **stability of the glucoamylase variant**. Although many peptide extensions are not believed to confer any substantial structural instability to the resulting glucoamylase variant, it may in certain instances and with certain parent glucoamylases be relevant to choose a peptide extension, which in itself can confer a structural stability to the modified glucoamylase enzyme. For instance, the peptide extension can increase the number of interactions and/or be covalently bound by adding cysteine bridges to from the N-terminal extension to the N-terminal residues as discussed below.

Brief Summary Text - BSTX (78):

While care must be exerted to select a proper expression system for producing a glucoamylase variant of the invention (in particular when a modified DNA sequence is used for the production), it has been found that a glucoamylase **variant according to the invention (having an improved thermal stability**) may be obtained by expressing a DNA sequence encoding the parent glucoamylase enzyme in question in an expression system which is incapable of processing the translated polypeptide in the normal manner, and thereby results in the production of an glucoamylase which comprises a part of or the entire propeptide or a similar peptide sequence associated with the mature protein prior to its processing. In this case, the propeptide or similar peptide sequence constitutes the peptide extension. The pro-peptide or similar peptide sequence may be heterologous or homologous to the parent glucoamylase and can be present in the N-terminal of the parent glucoamylase. The production of a glucoamylase variant according to the invention using this latter technique is described further below.

Brief Summary Text - BSTX (93):

In a preferred embodiment the **variant of the invention has improved thermal stability** within the temperature interval from about 60-80.degree. C., preferably 63-75.degree. C., at a pH of 4-5, in particular 4.2-4.7, using e.g. maltodextrin as the substrate.

Brief Summary Text - BSTX (108):

Random mutagenesis is suitably performed either as localised or region-specific random mutagenesis in at least three parts of the gene translating to the amino acid sequence shown in question, or within the whole gene. The random mutagenesis of a DNA sequence encoding a parent glucoamylase may be conveniently performed by use of any method known in the art. In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent glucoamylase, wherein the **variant exhibits increased thermal stability** relative to the parent, the method comprising:

Brief Summary Text - BSTX (111):

(c) screening for host cells expressing a glucoamylase **variant which has an altered property (i.e. thermal stability)** relative to the parent glucoamylase. Step (a) of the above method of the invention is preferably performed using doped primers, as described in the working examples herein (vide infra). For instance, the random mutagenesis may be performed by use of a suitable physical or chemical mutagenizing agent, by use of a suitable oligonucleotide, or by subjecting the DNA sequence to PCR generated mutagenesis. Furthermore, the random mutagenesis may be performed by use of any combination of these mutagenizing agents. The mutagenizing agent may, e.g., be one which induces transitions, transversions, inversions, scrambling, deletions, and/or insertions. Examples of a physical or chemical mutagenizing agent suitable for the present purpose include ultraviolet (UV) ir-radiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), O-methyl hydroxylamine, nitrous acid, ethyl methane sulphonate (EMS), sodium bisulphite, formic acid, and nucleotide analogues. When such agents are used, the mutagenesis is typically performed by incubating the DNA sequence encoding the parent enzyme to be mutagenized in the presence of the mutagenizing agent of choice under suitable conditions for the mutagenesis to take place, and selecting for mutated DNA having the desired properties. When the mutagenesis is performed by the use of an oligonucleotide, the oligonucleotide may be doped or spiked with the three non-parent nucleotides during the synthesis of the oligonucleotide at the positions which are to be changed. The doping or spiking may be done so that codons for unwanted amino acids are avoided. The doped or spiked oligonucleotide can be incorporated into the DNA encoding the glucoamylase enzyme by any published technique, using e.g. PCR, LCR or any DNA polymerase and ligase as deemed appropriate. Preferably, the doping is carried out using "constant random doping", in which the percentage of wild-type and mutation in each position is predefined. Furthermore, the doping may be directed toward a preference for the introduction of certain nucleotides, and thereby a preference for the introduction of one or more specific amino acid residues. The doping may be made, e.g., so as to allow for the introduction of 90% wild type and 10% mutations in each position. An additional consideration in the choice of a doping scheme is based on genetic as well as protein-structural constraints. The doping scheme may be made by using the DOPE program which, inter alia, ensures that introduction of stop codons is avoided. When PCR-generated mutagenesis is used, either a chemically treated or non-treated gene encoding a parent glucoamylase is subjected to PCR under conditions that increase the mis-incorporation of nucleotides (Deshler 1992; Leung et al., Technique, Vol.1, 1989, pp. 11-15). A mutator strain of E. coli (Fowler et al., Molec. Gen. Genet., 133, 1974, pp. 179-191), S. cereviseae or any other microbial organism may be used for the random mutagenesis of the DNA encoding the glucoamylase by, e.g., transforming a plasmid containing the parent glycosylase into the mutator strain, growing the mutator strain with the plasmid and isolating the mutated plasmid from the mutator strain. The mutated plasmid may be subsequently transformed into the expression organism. The DNA sequence to be mutagenized may be conveniently present in a genomic or cDNA library prepared from an organism expressing the parent glucoamylase. Alternatively, the DNA sequence may be present on a suitable vector such as a plasmid or a bacteriophage, which as such may be incubated with or other-wise exposed to the mutagenising agent. The DNA to be mutagenized may also be present in a host cell either by being integrated in the genome of said cell or by being present on a vector harboured in the cell. Finally, the DNA to be mutagenized may be in isolated form. It will be understood that the DNA

sequence to be subjected to random mutagenesis is preferably a cDNA or a genomic DNA sequence. In some cases it may be convenient to amplify the mutated DNA sequence prior to performing the expression step b) or the screening step c) . Such amplification may be performed in accordance with methods known in the art, the presently preferred method being PCR-generated amplification using oligonucleotide primers prepared on the basis of the DNA or amino acid sequence of the parent enzyme. Subsequent to the incubation with or exposure to the mutagenising agent, the mutated DNA is expressed by culturing a suitable host cell carrying the DNA sequence under conditions allowing expression to take place. The host cell used for this purpose may be one which has been transformed with the mutated DNA sequence, optionally present on a vector, or one which was carried the DNA sequence encoding the parent enzyme during the mutagenesis treatment. Examples of suitable host cells are the following: gram positive bacteria such as Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus, Bacillus megaterium, Bacillus thuringiensis, Streptomyces lividans or Streptomyces murinus; and gram-negative bacteria such as E. coli. The mutated DNA sequence may further comprise a DNA sequence encoding functions permitting expression of the mutated DNA sequence.

Brief Summary Text - BSTX (121):

Examples of suitable promoters for directing the transcription of the DNA sequence encoding a glucoamylase **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, the TPI (triose phosphate isomerase) promoter from S. cerevisiae (Alber et al. (1982), J. Mol. Appl. Genet 1, p. 419-434, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Brief Summary Text - BSTX (147):

Further, by improving the thermal stability the T.sub.1/2 (half-time, as defined in the "Materials and Methods" section) is improved. As the thermal **stability of the glucoamylase variants** of the invention is improved a minor amount of glucoamylase need to be added to replace the glucoamylase being inactivated during the saccharification process. More glucoamylase is maintained active during saccharification process according to the present invention. Furthermore, the risk of microbial contamination is also reduced when carrying the saccharification process at temperature above 63.degree. C.

Brief Summary Text - BSTX (182):

Thermal **Stability Determination f Variant** of the Invention

Brief Summary Text - BSTX (183):
The thermal **stability of variants** of the invention is tested using the following method: 950 microliter 50 mM sodium acetate buffer (pH 4.3) (NaOAc) is incubated for 5 minutes at 70.degree. C. 50 microliter enzyme in buffer (4 AGU/ml) is added. 2.times.40 microliter samples are taken at 0, 5, 20 and/or 40 minutes, respectively, and chilled on ice. The activity (AGU/ml) measured before incubation (0 minutes) is used as reference (100%). The decline in percent is calculated as a function of the incubation time.

Brief Summary Text - BSTX (219):
3. Decide on which kind of mutations should be carried out, e.g., with respect to the desired **stability and/or performance of the variant** to be constructed,

Detailed Description Text - DETX (65):
Glucoamylase **Variants with Increased Thermal Stability**

Detailed Description Text - DETX (69):
Glucoamylase **Variants with Increased Thermal Stability**

Detailed Description Text - DETX (70):
The thermal **stability activity of improved variants** expressed in yeast was measured on crude samples at pH 4.5, 68.degree. C., as described in Methods section above.

Detailed Description Text - DETX (72):
Glucoamylase **Variants with Increased Thermal Stability**

Detailed Description Text - DETX (73):
The thermal **stability activity of improved variants** expressed in A. niger was measured on crude samples at pH 4,5, 70.degree. C. as described in Methods section above.

Claims Text - CLTX (60):
28. The variant glucoamylase of claim 1, wherein the **variant has improved thermal stability** compared to the parent glucoamylase.

US-PAT-NO: 6297038

DOCUMENT-IDENTIFIER: US 6297038 B1

TITLE: Amylase variants

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen N	N/A	N/A	DK

APPL-NO: 09/ 354191

DATE FILED: July 15, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 08/600,656 filed on Feb. 13, 1996 now U.S. Pat. No. 6,093,562 which is a continuation of application Ser. No. PCT/DK96/00056 filed on Feb. 5, 1996, and claims priority under 35 U.S.C. 119 of Danish application serial nos. 0126/95 filed on Feb. 3, 1995; 0336/95 filed on Mar. 29, 1995; 1097/95 filed on Sep. 29, 1995; and 1121/95 filed on Oct. 6, 1995, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0126/95	February 3, 1995
DK	0336/95	March 29, 1995
DK	1097/95	September 29, 1995
DK	1121/95	October 6, 1995

US-CL-CURRENT: 435/202

ABSTRACT:

The present invention relates to variants of a parent .alpha.-amylase, which parent .alpha.-amylase (i) has an amino acid sequence selected from the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 7, respectively; or (ii) displays at least 80% homology with one or more of these amino acid sequences; and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of these amino acid sequences; and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an a-amylase having one of these amino acid sequences; in which variant:

(a) at least one amino acid residue of the parent .alpha.-amylase has been deleted; and/or

(b) at least one amino acid residue of the parent .alpha.-amylase has been replaced by a different amino acid residue; and/or

(c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency;

with the proviso that the amino acid sequence of the variant is not identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No.3 and SEQ ID No. 7, respectively.

57 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (9):
WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent (.alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Brief Summary Text - BSTX (13):
EP 525 610 relates to **mutant enzymes having improved stability** towards ionic tensides (surfactants). The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations are not specified.

Brief Summary Text - BSTX (14):
WO 94/02597 discloses .alpha.-amylase **mutants which exhibit impr ved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino

acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Brief Summary Text - BSTX (17):

An object of the present invention is to provide .alpha.-amylase variants which--relative co their parent .alpha.-amylase--possess improved properties of importance, inter alia, in relation to the washing and/or dishwashing performance of the **variants in question, e.g. increased thermal stability,** increased stability towards oxidation, reduced dependency on Ca.sup.2+ ion and/or improved stability or activity in the pH region of relevance in, e.g., laundry washing or dishwashing. Such variant .alpha.-amylases have the advantage, among others, that they may be employed in a lower dosage than their parent .alpha.-amylase. Furthermore, the .alpha.-amylase variants may be able to remove starchy stains which cannot, or can only with difficulty, be removed by .alpha.-amylase detergent enzymes known today.

Brief Summary Text - BSTX (92):

From the results obtained by the present inventors it appears that changes in a particular property, e.g. thermal **stability or oxidation stability, exhibited by a variant** relative to the parent .alpha.-amylase in question can to a considerable extent be correlated with the type of, and positioning of, mutation(s) (amino acid substitutions, deletions or insertions) in the variant. It is to be understood, however, that the observation that a particular mutation or pattern of mutations leads to changes in a given property in no way excludes the possibility that the mutation(s) in question can also influence other properties.

Brief Summary Text - BSTX (93):

Oxidation stability: With respect to increasing the oxidation **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable that at least one, and preferably multiple, oxidizable amino acid residue(s) of the parent has/have been deleted or replaced (i.e. substituted by) a different amino acid residue which is less susceptible to oxidation than the original oxidizable amino acid residue.

Brief Summary Text - BSTX (94):

Particularly relevant oxidizable amino acid residues in this connection are cysteine, methionine, tryptophan and tyrosine. Thus, for example, in the case of parent .alpha.-amylases containing cysteine it is anticipated that deletion of cysteine residues, or substitution thereof by less oxidizable amino acid residues, will be of importance in obtaining **variants with improved oxidation stability** relative to the parent .alpha.-amylase.

Brief Summary Text - BSTX (95):

In the case of the above-mentioned parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, respectively, all of which contain no cysteine residues but have a significant

methionine content, the deletion or substitution of methionine residues is particularly relevant with respect to achieving improved oxidation **stability of the resulting variants**. Thus, deletion or substitution [e.g. by threonine (T), or by one of the other amino acids listed above] of one or more of the methionine residues in positions M9, M10, M105, M202, M208, M261, M309, M382, M430 and M440 of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, and/or in position M323 of the amino acid sequence shown in SEQ ID No. 2 (or deletion or substitution of methionine residues in equivalent positions in the sequence of another .alpha.-amylase meeting one of the other criteria for a parent .alpha.-amylase mentioned above) appear to be particularly effective with respect to increasing the oxidation stability.

Brief Summary Text - BSTX (104):

Thermal stability: With respect to increasing the thermal **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ ID No. 1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively, are: F180, R181, G182, D183, G184 and K185 (SEQ ID No. 2); F178, R179, G180, I181, G182 and K183 (SEQ ID No. 3); and F180, R181, G182, H183, G184 and K185 (SEQ ID No. 7).

Brief Summary Text - BSTX (111):

Examples of specific mutations which appear to be of importance in connection with the thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequence shown in SEQ ID No. 1 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): K269R; P260E; R124P; M105F,I,L,V; M208F,W,Y; L217I; V206I,L,F.

Brief Summary Text - BSTX (115):

Still further examples of mutations which appear to be of importance, inter alia, in achieving improved thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): A354C+V479C; L351C+M430C; N457D,E+K385R; L355D,E+M430R,K; L355D,E+I411R,K; and N457D,E.

Brief Summary Text - BSTX (208):

.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen

to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 1 can barely be detected. Stabilized variants show, under the same conditions, increased color intensity due to increased liberation of Cibacron Red.

Brief Summary Text - BSTX (218):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** lichenifonmis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothernophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulians acetamidase.

Detailed Description Text - DETX (103):

Determination of Oxidation **Stability of M202 Substitution Variants** of the Parent .alpha.-amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detailed Description Text - DETX (104):

A: Oxidation **Stability of Variants** of the Sequence in SEQ ID No. 1

Detailed Description Text - DETX (109):

B: Oxidation **Stability of Variants** of the Sequence in SEQ ID No. 2

Detailed Description Text - DETX (113):

Determination of Thermal **Stability of Variants** of the Parent .alpha.-amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detailed Description Text - DETX (114):

A: Thermal **Stability of Pairwise Deletion Variants** of the Sequence in SEQ ID No. 1

Detailed Description Text - DETX (122):

It is apparent that all of the pairwise deletion variants tested exhibit significantly improved thermal stability relative to the parent .alpha.-amylase (SEQ ID No. 1), and that the thermal **stability of Variant** 5, which in addition to the pairwise deletion mutation of Variant 4 comprises the substitution R124P, is markedly higher than that of the other variants. Since calorimetric results for the substitution variant R124P (comprising only the substitution R124P) reveal an approximately 7.degree. C. thermostabilization thereof relative to the parent .alpha.-amylase, it appears that the thermostabilizing effects of the mutation R124P and the pairwise deletion, respectively, reinforce each other.

Detailed Description Text - DETX (123):

B: Thermal **Stability of Pairwise Deletion Variants** of the Sequence in SEQ ID No. 2

Detailed Description Text - DETX (129):

Thermal **Stability of a Multi-combination Variant** of the Sequence in SEQ ID No. 1

US-PAT-NO: 6297037

DOCUMENT-IDENTIFIER: US 6297037 B1
See image for Certificate of Correction

TITLE: Oxidatively stable alpha-amylase

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	94080	N/A
Mitchinson; Colin	Half Moon Bay	CA	94019	N/A
Power; Scott D.	San Bruno	CA	94066	N/A
Requadt; Carol A.	Tiburon	CA	94920	N/A

APPL-NO: 08/ 194664

DATE FILED: February 10, 1994

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993, now abandoned.

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/274 , 435/275 , 435/471 , 435/485 , 510/226 , 510/320 , 510/392 , 510/393

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

25 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

Brief Summary Text - BSTX (2):

The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The **mutant enzymes of the present invention exhibit altered stability**/activity profiles including but not limited to altered oxidative stability, altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (13):

The alpha-amylase **mutants of the present invention, in general, exhibit altered oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. **Mutant enzymes having enhanced oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced oxidative stability may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby **mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability** at high pH cleaning product conditions. The **mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability** at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase .

Brief Summary Text - BSTX (15):

The preferred **alpha-amylase mutants** of the present invention are derived from a **Bacillus** strain such as B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus, and most preferably from **Bacillus** licheniformis.

Drawing Description Text - DRTX (14):
FIG. 10 shows thermal **stability f M197X variants** at pH 5.0, 5 mM CaCl.sub.2 at 95.degree. C. for 5 mins.

Drawing Description Text - DRTX (19):
FIG. 15 shows heat **stability of M15X variants** at 90.degree. C., pH 5.0, 5 mM CaCl.sub.2, 5 mins.

Detailed Description Text - DETX (10):
Host strains (or cells) useful in the present invention generally are procaryotic or eucaryotic hosts and include any transformable microorganism in which the expression of **alpha-amylase** can be achieved. Specifically, host strains of the same species or genus from which the **alpha-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably an **alpha-amylase** negative **Bacillus** strain (genes deleted) and/or an **alpha-amylase** and protease deleted **Bacillus** strain such as **Bacillus** subtilis strain BG2473 (.DELTA.amyE,.DELTA.apr,.DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **alpha-amylase and its variants (mutants)** or expressing the desired **alpha-amylase**.

Detailed Description Text - DETX (84):
All M15X variants were propagated in Bacillus subtilis and the expression level monitored as shown in FIG. 13. The amylase was isolated and partially purified by a 20-70% ammonium sulfate cut. The specific activity of these variants on the soluble substrate was determined as per Example 3 (FIG. 14). Many of the M15X amylases have specific activities greater than that of Spezyme.RTM. AA20. A benchtop heat **stability assay was performed on the variants** by heating the amylase at 90.degree. C. for 5 min. in 50 mM acetate buffer pH 5 in the presence of 5 mM CaCl.sub.2 (FIG. 15). Most of the variants performed as well as Spezyme.RTM. AA20 in this assay. Those **variants that exhibited reasonable stability** in this assay (reasonable stability defined as those that retained at least about 60% of Spezyme.RTM. AA20's heat stability) were tested for specific activity on starch and for liquefaction performance at pH 5.5. The most interesting of those mutants are shown in FIG. 16. M15D, N and T, along with L, outperformed Spezyme.RTM. AA20 in liquefaction at pH 5.5 and have increased specific activities in both the soluble substrate and starch hydrolysis assays.

Claims Text - CLTX (1):
1. A **mutant alpha amylase** derived from **Bacillus, the mutant alpha amylase** having a substitution of an amino acid selected from the group consisting of threonine, leucine, alanine, arginine, asparagine, aspartic acid, glutamic acid, glycine, isoleucine, lysine, phenylalanine, proline, serine, valine, histidine and glutamine or a deletion of a methionine residue at a position

equivalent to M197 **Bacillus** licheniformis **alpha amylase**.

Claims Text - CLTX (4):
4. A **mutant alpha-amylase** of claim 3 wherein the precursor is **Bacillus** licheniformis **alpha-amylase**.

Claims Text - CLTX (9):
9. A **mutant alpha amylase** derived from **Bacillus, the mutant alpha amylase** having a substitution of an amino acid selected from the group consisting of leucine, threonine, asparagine, aspartate, serine, valine, and isoleucine for a methionine residue at a position equivalent to M15 in **Bacillus** licheniformis **alpha amylase**.

Claims Text - CLTX (12):
12. A **mutant alpha-amylase** of claim 9 further comprising the substitution or deletion of an amino acid residue in the precursor **alpha amylase** at a position equivalent to either or both of M197 or W138 in **Bacillus** licheniformis **alpha-amylase**.

Claims Text - CLTX (13):
13. A **mutant alpha-amylase** of claim 12 further comprising substitutions equivalent to M197T/W138F or M197T/W138Y in **Bacillus** licheniformis **alpha-amylase**.

Claims Text - CLTX (23):
23. A **mutant alpha amylase** derived from **Bacillus, the mutant alpha amylase** having at least two substitutions at positions equivalent to M15, W138 and/or M197 in **Bacillus** licheniformis **alpha amylase,** wherein said substitutions comprise M15T, W138Y and/or M197T.

Claims Text - CLTX (24):
24. A **mutant alpha-amylase having enhanced oxidative stability, the mutant alpha-amylase** being derived from **Bacillus** and comprising a substitution of methionine with alanine, arginine, glycine, lysine, phenylalanine, proline, threonine or valine at an amino acid residue equivalent to M197 in **Bacillus** licheniformis **alpha-amylase**.

Claims Text - CLTX (25):
25. A **mutant alpha-amylase** according to claim 24, said substitution comprising substituting methionine with threonine at an amino acid residue equivalent to M197 in **Bacillus** licheniformis **alpha-amylase**.

US-PAT-NO: 6265197

DOCUMENT-IDENTIFIER: US 6265197 B1

TITLE: Starch debranching enzymes

DATE-ISSUED: July 24, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK

APPL-NO: 09/ 346237

DATE FILED: July 1, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish application PA 1998 00868 filed Jul. 2, 1998 and Provisional application 60/094,353 filed Jul. 28, 1998, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1998 00868	July 2, 1998

US-CL-CURRENT: 435/210, 435/183 , 435/200

ABSTRACT:

The invention relates to a genetically engineered variant of a parent starch debranching enzyme, i.e. a pullulanase or an isamylase, the enzyme variant having an improved thermostability at a pH in the range of 4-6 compared to the parent enzyme and/or an increased activity towards amylopectin and/or glycogen compared to the parent enzyme, to methods for producing such starch debranching enzyme variants with improved thermostability and/or altered substrate specificity, and to a method for converting starch to one or more sugars using at least one such enzyme variant.

8 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (33):
Examples of specific **.alpha.-amylases** which can be used in the liquefaction step include **Bacillus** licheniformis **.alpha.-amylases,** such as the commercially available products Termamyl.RTM., Spezyme.RTM. AA, Spezyme.RTM. Delta AA, Maxamyl.RTM. and Kleistase.RTM., and the **.alpha.-amylase mutants** described in WO 96/23874 (Novo Nordisk) and PCT/DK97/00197 (Novo Nordisk).

Detailed Description Text - DETX (112):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent enzyme, wherein the **variant exhibits improved thermal stability** relative to the parent, the method comprising:

Detailed Description Text - DETX (115):
(c) screening for host cells expressing an enzyme **variant which has an altered property (e.g. thermal stability**) relative to the parent enzyme.

US-PAT-NO: 6242406

DOCUMENT-IDENTIFIER: US 6242406 B1
See image for Certificate of Correction

TITLE: Mid-chain branched surfactants with cellulose derivatives

DATE-ISSUED: June 5, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Katsuda; Rinko	Kobe	N/A	N/A	JP
Kawasaki; Eriko	Kobe	N/A	N/A	JP
Murata; Susumu	Nishinomiya	N/A	N/A	JP

APPL-NO: 09/ 529261

DATE FILED: April 10, 2000

PCT-DATA:
APPL-NO: PCT/US97/18841
DATE-FILED: October 10, 1997
PUB-NO: WO99/19445
PUB-DATE: Apr 22, 1999
371-DATE: Apr 10, 2000
102(E)-DATE:Apr 10, 2000

US-CL-CURRENT: 510/357, 510/424 , 510/426 , 510/427 , 510/428 , 510/473

ABSTRACT:

Mid-chain branched surfactants derived from mid-chain branched primary alkyl hydrophobic groups and hydrophilic groups. The present invention also relates to mixtures of mid-chain branched surfactants useful in laundry and cleaning compositions, especially granular and liquid detergent compositions.

18 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (92):
Amylases suitable herein, especially for, but not limited to automatic dishwashing purposes, include, for example, a-amylases described in GB 1,296,839 to Novo; RAPIDAS.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful.

Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, Jun. 1985, pp. 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents such as automatic dishwashing types, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the **Bacillus** amylases, especially the **Bacillus** a-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the aboveidentfied reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is rmade, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. lichenifonnis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtils, or B. stearothemnophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant alpha-Arnylases" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformnis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M 197T with the M197T **variant being the most stable expressed variant Stability** was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6211134

DOCUMENT-IDENTIFIER: US 6211134 B1
See image for Certificate of Correction

TITLE: Mutant .alpha.-amylase

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Caldwell; Robert M.	San Carlos	CA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Ropp; Traci H	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 985659

DATE FILED: December 9, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of U.S. Ser. No. 08/645,971 filed on May 14, 1996 U.S. Pat. No. 5,763,385.

US-CL-CURRENT: 510/392, 510/226 , 510/321 , 510/330

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed having a substution equivalent to G475R in Bacillus licheniformis. The disclosed .alpha.-amylase enzymes show improved specific activity and starch hydrolysis performance. Also provided are polynucleotides encoding such enzymes, expression vectors including such polynucleotides, host cells transformed with such expression vectors, and the use of such enzymes in detergent compositions.

17 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Brief Summary Text - BSTX (16):
In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having impr ved xidative stability** is described wherein one or more methionines are replaced

by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (17):
In PCT publication No. WO 94/18314, a **mutant .alpha.-amylase having impr ved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Drawing Description Text - DRTX (10):
FIG. 9 illustrates a schematic of the PCR method used to produce the **mutant** oligonucleotides corresponding to **.alpha.-amylase** derived from **Bacillus** licheniformis.

Detailed Description Text - DETX (5):
"Host strain" or "host cell" means a suitable host for an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (.DELTA.amyE, .DELTA.apr, .notident.npr) is used. Host cells are transformed or transfected with vectors constructed using common techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (13):
The **.alpha.-amylases** according to the present invention exhibit improved specific activity and liquefaction performance providing desirable and unexpected results which are useful in the various applications for which **.alpha.-amylases** are commonly used. The **.alpha.-amylase** of the present invention is especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring **.alpha.-amylases** exhibiting improved low pH performance, improved thermal stability and improved oxidative stability. Accordingly, **.alpha.-amylases** according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably between about 5.0 and 5.5. Additionally, **.alpha.-amylases** according to the present invention which exhibit increased thermal stability at temperatures of between about 80-120.degree. C., and preferably between about 100-110.degree. C., and increased stability in the presence of oxidants will be particularly useful. Preferably, the **.alpha.-amylase** according to the present invention which is used in liquefaction, in addition to substitution of a residue corresponding to G475, further comprises a deletion or substitution at one or more residues

corresponding to M15, V128, H133, W138, V148, S187, M197, A209 and/or A379 in **Bacillus** licheniformis. Most preferably, the amylase comprises a substitution corresponding to M15T/H133Y/V148S/N188SA209V/A379S/G475R in **Bacillus** licheniformis. In any event, because it is contemplated that many mutations provide incremental advantages, the combination of such a mutation with the **mutants** of the invention should provide additive benefits. Thus, for example, because a mutation corresponding to M197T has been established as providing exceptional oxidation **stability, the addition of a M197T modification to a mutant .alpha.-amylase** of the invention should provide a similar boost in oxidative stability.

Detailed Description Text - DETX (17):

Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Pat. No. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Pat. No. Re. 34,606.

Detailed Description Text - DETX (19):

A wide variety of host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of E. coli, Pseudomonas, **Bacillus,** Streptomyces, various fungi, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (20):

The improved **.alpha.-amylases** according to the present invention provide several important advantages when compared to wild type **Bacillus .alpha.-amylases**. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and oxidative stability which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of **.alpha.-amylase** according to the invention provide superior performance when compared to wild type **Bacillus** licheniformis **.alpha.-amylase** due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater

potential performance benefits of this **variant**. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

Detailed Description Text - DETX (45):

Transformation Of Plasmids Into **Bacillus** subtilis, Expression And Purification of **Mutant .alpha.-Amylase**

Detailed Description Text - DETX (46):

.alpha.-Amylase is expressed in **Bacillus** subtilis after transformation with the plasmids described in Examples 1-3. pHP13 is a plasmid able to replicate in E. coli and in **Bacillus** subtilis. Plasmids containing different **variants** are constructed using an appropriate E. coli strain, e.g., E. coli MM294. The plasmids isolated and then transformed into **Bacillus** subtilis as described in Anagnostopoulos et al., J. Bacter., Vol. 81, pp. 741-746 (1961). The **Bacillus** strain is deleted for two proteases (.DELTA.apr, .DELTA.npr) (see e.g., Ferrari et al., U.S. Pat. No. 5,264,366) and for amylase (.DELTA.amyE) (see e.g., Stahl et al., J. Bacter., Vol. 158, pp. 411-418 (1984)). After transformation, the sacU(Hy) mutation (Henner et al., J. Bacter., Vol., 170, pp. 296-300 (1988)) is introduced by PBS-1 mediated transduction (Hoch,, J. Bacter., Vol. 154, pp. 1513-1515 (1983)).

US-PAT-NO: 6204232

DOCUMENT-IDENTIFIER: US 6204232 B1

TITLE: .alpha.-amlase mutants

DATE-ISSUED: March 20, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchert; Torben Vedel	Copenhagen	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Andersen; Carsten	Vaerloese	N/A	N/A	DK
Nielsen; Bjarne	Virum	N/A	N/A	DK
Nissen; Torben Lauesgaard	Frederiksberg	N/A	N/A	DK
Kj.ae butted.rulff; S.o slashed.ren	Vanl.o slashed.se	N/A	N/A	DK

APPL-NO: 09/ 183412

DATE FILED: October 30, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish application Ser. Nos. 1240/97 filed Oct. 30, 1997 and PA1998 00936 filed on Jul. 14, 1998, and U.S. Provisional application serial Nos. 60/064,662 filed on Nov. 6, 1997 and 60/093,234 filed on Jul. 17, 1998, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1240/97	October 30, 1997
DK	1998 00936	July 14, 1998

US-CL-CURRENT: 510/226, 435/202 , 510/236 , 510/320 , 510/396

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, which exhibits an alteration in at least one of the following properties relative to said parent .alpha.-amylase: i) improved pH stability at a pH from 8 to 10.5; and/or ii) improved Ca.sup.2+ stability at pH 8 to 10.5, and/or iii) increased specific activity at temperatures from 10 to 60.degree. C.

31 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Brief Summary Text - BSTX (6):
Among more recent disclosures relating to **.alpha.-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** (BAN.TM.) and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens (BAN.TM.) and B. stearothermophilus (BSG.TM.) **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (14):
Alterations in properties which may be achieved in **variants(mutants) of the invention are alterations in: the stability** of the Termamyl-like .alpha.-amylase at a pH from 8 to 10.5, and/or the Ca.sup.2+ stability at pH 8 to 10.5, and/or the specific activity at temperatures from 10 to 60.degree. C., preferably 20-50.degree. C., especially 30-40.degree. C.

Detailed Description Text - DETX (48):
Preferred high pH **stability variants** include one or more of the following substitutions in the SP722 .alpha.-amylase (having the amino acid sequence shown in SEQ ID NO: 2):

Detailed Description Text - DETX (52):
.alpha.-amylase **variants with improved stability** at high pH can be constructed by making substitutions in the regions found using the molecular dynamics simulation mentioned in Example 2. The simulation depicts the region(s) that has a higher flexibility or mobility at high pH (i.e., pH 8-10.5) when compared to medium pH.

Detailed Description Text - DETX (76):
In a preferred embodiment the **variant is the Bacillus** strain NCIB 12512 **.alpha.-amylase** with deletions in D183 and G184 and further one of the following substitutions: R181Q,N and/or G182T,S,N and/or D183*; G184* and/or

K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V and/or A186T,S,N,I,V and/or W189T,S,N,Q and/or N195F and/or N270R,D and/or E346Q and/or K385R and/or K458R and/or P459T.

Detailed Description Text - DETX (171):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E.coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyL), the promoters of the **Bacillus** amiyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (206):

The assay can be used to screening of Termamyl-like .alpha.-amylase **variants having an improved stability** at high pH compared to the parent enzyme and Termamyl-like .alpha.-amylase **variants having an improved stability** at high pH and medium temperatures compared to the parent enzyme depending of the screening temperature setting

Detailed Description Text - DETX (237):

3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed

Detailed Description Text - DETX (261):

Method of Extracting Important Regions for Identifying .alpha.-amylase **Variants with Improved pH Stability** and Altered Temperature Activity

Detailed Description Text - DETX (264):

1. The approach used for extracting important regions for identifying .alpha.-amylase **variants with high pH stability**:

Detailed Description Text - DETX (265):

The important regions for constructing **variants with improved pH stability** are the regions which at the extreme pH display the highest mobility, i.e.,

regions having the highest isotropic fluctuations.

Detailed Description Text - DETX (272):
Construction, by Localized Random, Doped Mutagenesis, of Termamyl-like .alpha.-amylase **Variants Having an Improved Ca.sup.2+ Stability** at Medium Temperatures Compared to the Parent Enzyme

Detailed Description Text - DETX (394):
Determination of pH **Stability at Alkaline pH of Variants** of the Parent .alpha.-Amylase Having the Amino Acid Sequence Shown in SEQ ID NO:2.

Detailed Description Text - DETX (402):
Determination of Calcium **Stability at Alkaline pH of Variants** of the Parent .alpha.-Amylase Having the Amino Acid Sequence Shown in SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 4.

Detailed Description Text - DETX (403):
A: Calcium **Stability of Variants** of the Sequence in SEQ ID NO: 1

Detailed Description Text - DETX (407):
B: Calcium **Stability of Variants** of the Sequence in SEQ ID NO: 2

Detailed Description Text - DETX (414):
C: Calcium **Stability of Variants** of the Sequence in SEQ ID NO: 4

Claims Text - CLTX (14):
5. The **variant according to claim 1, wherein said variant exhibits improved stability** at pH 8 to 10.5.

Claims Text - CLTX (21):
8. The variant according to claim 1, wherein said **variant exhibits improved Ca.sup.2+ stability** at pH 8 to 10.5.

US-PAT-NO: 6197565

DOCUMENT-IDENTIFIER: US 6197565 B1

TITLE: .alpha.-Amylase variants

DATE-ISSUED: March 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkerod	N/A	N/A	DK
Kjaerulff; Soeren	Vanlose	N/A	N/A	DK
Bisgaard-Frantzen; Henrik	Bagsvaerd	N/A	N/A	DK
Andersen; Carsten	Vaerloese	N/A	N/A	DK

APPL-NO: 09/ 193068

DATE FILED: November 16, 1998

US-CL-CURRENT: 435/202, 435/204 , 435/210 , 510/226 , 510/236 , 510/320 , 510/392 , 510/530

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, comprising mutations in two, three, four, five or six regions/positions. The **variants have increased stability** at high temperatures (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased.

21 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Abstract Text - ABTX (1):

The invention relates to a variant of a parent Termamyl-like

.alpha.-amylase, comprising mutations in two, three, four, five or six regions/positions. The **variants have increased stability** at high temperatures (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased.

Brief Summary Text - BSTX (2):

The present invention relates, inter alia, to novel variants (mutants) of parent Termamyl-like .alpha.-amylases, notably **variants exhibiting increased stability** at acidic pH at low calcium concentrations and/or high temperatures (relative to the parent) which are advantageous with respect to applications of the variants in, in particular, industrial starch processing (e.g., starch liquefaction or saccharification).

Brief Summary Text - BSTX (6):

Among more recent disclosures relating to **.alpha.-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (8):

The present invention relates to novel .alpha.-amylolytic variants (mutants) of a Termamyl-like .alpha.-amylase, in particular **variants exhibiting increased stability** at acidic pH at high temperatures (relative to the parent) which are advantageous in connection with the industrial processing of starch (starch liquefaction, saccharification and the like).

Detailed Description Text - DETX (41):

The inventors have found that the above mentioned **variants have increased stability** at pHs below 7.0 (i.e., acidic pH) and/or at calcium concentration below 1 mM (40 ppm) (i.e, low calcium concentrations) at temperatures in the

range from 95 to 160.degree. C. (i.e., high temperatures) relative to the parent Termamyl-like .alpha.-amylase.

Detailed Description Text - DETX (71):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent .alpha.-amylase, e.g., wherein the **variant exhibits altered or increased thermal stability** relative to the parent, the method comprising:

Detailed Description Text - DETX (74):
(c) screening for host cells expressing an .alpha.-amylase **variant which has an altered property (i.e. thermal stability)** relative to the parent .alpha.-amylase.

Detailed Description Text - DETX (94):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (121):
LE174 hybrid **alpha-amylase variant**: LE174 is a hybrid Termamyl-like **alpha-amylase** being identical to the Termamyl sequence, i.e., the **Bacillus** licheniformis **.alpha.-amylase** shown in SEQ ID NO: 4, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the **Bacillus** amyloliquefaciens **alpha-amylase** shown in SEQ ID NO: 5, which further have following mutations: H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 4).

Detailed Description Text - DETX (148):
Construction, by random mutagenesis, of Termamyl-like LE174 .alpha.-amylase **variants having an improved stability** at low pH and a reduced dependency on calcium ions for stability compared to the parent enzyme

Detailed Description Text - DETX (161):

Normally, industrial liquefaction processes is run at pH 6.0-6.2 with addition of about 40 ppm free calcium in order to improve the stability at 95.degree. C.-105.degree. C. **Variants of the invention have been made in order to improve the stability** at

Claims Text - CLTX (2):

2. The **variant** of claim 1, wherein the parent Termamyl-like **.alpha.-amylase** is derived from a strain of B. licheniformis, B. amyloliquefaciens, B. stearothermophilus, **Bacillus** sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375.

US-PAT-NO: 6197070

DOCUMENT-IDENTIFIER: US 6197070 B1
See image for Certificate of Correction

TITLE: Detergent compositions comprising alpha combination of .alpha.-amylases for malodor stripping

DATE-ISSUED: March 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Horner; Thomas Wilhelm	Overijse	N/A	N/A	BE
Govers; Kristien Greta	Kontich	N/A	N/A	BE

APPL-NO: 09/ 180679

DATE FILED: November 13, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
WO	PCT/US96/07090	May 15, 1996

PCT-DATA:
APPL-NO: PCT/US97/08103
DATE-FILED: May 13, 1997
PUB-NO: WO97/43385
PUB-DATE: Nov 20, 1997
371-DATE: Nov 13, 1998
102(E)-DATE:Nov 13, 1998

US-CL-CURRENT: 8/137, 134/25.2 , 134/25.3 , 134/42 , 435/201 , 435/202 , 435/203 , 435/204 , 510/220 , 510/221 , 510/226 , 510/235 , 510/238 , 510/276 , 510/300 , 510/320 , 510/321 , 510/337 , 510/344 , 510/392 , 510/393 , 510/405 , 510/530 , 510/531

ABSTRACT:

Fabrics are laundered in detergent compositions containing a mixture of .alpha.-amylase enzymes to remove malodorous materials.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (31):

(ii) a **variant .alpha.-amylase** according to (i) wherein the oxidisable amino acid residue to be deleted or substitued is a Met in the precursor **.alpha.-amylase** equivalent to +8, +15, +197, +256, +304, +366 or +438 in **Bacillus** licheniformis **.alpha.-amylase.**

Brief Summary Text - BSTX (37):

(d) WO95/26397 Novo Nordisk, published Oct. 5, 1995 describes other suitable amylases: .alpha.-amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl.RTM. at a temperature range of 25.degree. C. to 55.degree. C. and at a pH value in the range of 8 to 10, measured by the Phadebas.RTM. .alpha.-amylase activity assay. Such Phadebas.RTM. .alpha.-amylase activity assay is described at pages 9-10, WO95/26397. Variants of these new amylases demonstrating at least one of the following properties relative to the parent enzymes: 1) improved thermal stability, 2) oxidation stability and 3) reduced calcium ion dependency properties. Examples of other desirable improvements or modifications of properties (relative to the parent-.alpha.-amylase) which may be achieved with a **variant according to the present invention are: increased stability** and/or .alpha.-amylolytic activity at neutral to relatively high pH values, increased .alpha.-amylolytic activity at relatively high temperature and increase or decrease of the isoelectric point (pI) so as to better match the pI value for .alpha.-amylase variant to the pH of the medium, have been described in the co-pending application by Novo Nordisk WO96/23873.

Brief Summary Text - BSTX (42):

Preferred combinations of .alpha.-amylases are combinations of a non-modified .alpha.-amylase derived from B. licheniformis, B. amyloliquefaciens, B. Subtilis or B. stearothermophilus with a .alpha.-amylase as described herein above under (a)-(c) and/or WO95/26397 and/or WO95/35382. More preferred combination are the combination of an .alpha.-amylase as described in WO95/10603, known as Duramyl.RTM. and the .alpha.-amylase derived from B. licheniformis, known as Termamyl.RTM. or with a variant of WO95/26397 .alpha.-amylases, preferably a **variant demonstrating improved thermal stability,** as described in WO96/23873. Other more preferred combinations are the combination of the .alpha.-amylase derived from B. licheniformis, known as Termamyl.RTM. with a variant of WO95/26397 .alpha.-amylases, preferably demonstrating improved thermal stability, as described in WO96/23873; with a Purafact OX AM.RTM. (WO94/18314) and/or with the parent hybrid .alpha.-amylase described in WO96/23874. The detergent compositions of the present invention can comprise a combination of more than two .alpha.-amylases.

Claims Text - CLTX (3):

(b) an **.alpha.-amylase variant** comprising a C-terminal part of an **.alpha.-amylase** derived from **Bacillus** licheniformis and a N-terminal part of an **.alpha.-amylase** derived from **Bacillus** amyloliquefaciens or from **Bacillus** stearothermophilus wherein the Met amino acid residue at position 197 has been substituted by a Leu, Thr, Ala, Gly, Ser, Ile or Asp amino acid residue, said laundering solution having a temperature of from 5.degree. C. to 95.degree. C. and a pH of 7 to 11.

US-PAT-NO: 6187576

DOCUMENT-IDENTIFIER: US 6187576 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: February 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Borchert; Torben Vedel	Jyllinge	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK

APPL-NO: 09/ 170670

DATE FILED: October 13, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

The application claims priority under 35 U.S.C 119 of Danish application 1172/97 filed Oct. 13, 1997, and of U.S. provisional application 60/063,306 filed Oct. 28, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1172/97	October 13, 1997

US-CL-CURRENT: 435/202, 435/183 , 435/200 , 510/226 , 510/235 , 510/320 , 510/392

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, comprising mutations in two, three, four, five or six regions/positions. The variants have increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase variant of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase variant of the invention, a manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant of the invention, a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which variant exhibits increased thermostability at acidic pH and/or at low Ca.sup.2+ concentrations (relative

to the parent).

22 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

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Brief Summary Text - BSTX (6):
Among more recent disclosures relating to **.alpha.-amylases**, WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Detailed Description Text - DETX (252):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent .alpha.-amylase, e.g. wherein the **variant exhibits altered or increased thermal stability** relative to the parent, the method comprising:

Detailed Description Text - DETX (255):
(c) screening for host cells expressing an .alpha.-amylase **variant which has an altered property (i.e. thermal stability)** relative to the parent .alpha.-amylase.

Detailed Description Text - DETX (275):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus**

stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amiyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (306):

BSG **alpha-amylase**: B. stearothermophilus **alpha-amylase** depicted in SEQ ID NO: 3. TVB146 **alpha-amylase variant**: B. stearothermophilus **alpha-amylase variant** depicted in SEQ ID NO: 3 with the following mutations: with the deletion in positions I181-G182+N193F. LE174 hybrid **alpha-amylase variant**: LE174 is a hybrid Termamyl-like **alpha-amylase** being identical to the Termamyl sequence, i.e., the **Bacillus** licheniformis **.alpha.-amylase** shown in SEQ ID NO: 4, except that the N-terminal 35 amino acid residues (of the mature protein) has been replaced by the N-terminal 33 residues of BAN (mature protein), i.e., the **Bacillus** amyloliquefaciens **alpha-amylase** shown in SEQ ID NO: 5, which further have following mutations: H156Y+A181T+N190F+A209V+Q264S (using the numbering in SEQ ID NO: 4). LE174 was constructed by SOE-PCR (Higuchi et al. 1988, Nucleic Acids Research 16:7351).

US-PAT-NO: 6184188

DOCUMENT-IDENTIFIER: US 6184188 B1

TITLE: Fragrance delivery system for liquid detergent compositions

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Severns; John Cort	West Chester	OH	N/A	N/A
Sivik; Mark Robert	Fairfield	OH	N/A	N/A
Costa; Jill Bonham	Cincinnati	OH	N/A	N/A
Hartman; Frederick Anthony	Cincinnati	OH	N/A	N/A
Morelli; Joseph Paul	Cincinnati	OH	N/A	N/A

APPL-NO: 09/ 242650

DATE FILED: March 18, 1999

PARENT-CASE:

CROSS REFERENCE

This application claims priority under Title 35, United States Code 119(e) from Provisional Application Serial No. 60/024,117, filed Aug. 19, 1996.

PCT-DATA:
APPL-NO: PCT/US97/14664
DATE-FILED: August 19, 1997
PUB-NO: WO98/07814
PUB-DATE: Feb 26, 1998
371-DATE: Mar 18, 2000
102(E)-DATE:Mar 18, 2000

US-CL-CURRENT: 510/101, 510/107 , 510/337 , 510/338 , 510/505

ABSTRACT:

This invention is a fabric delivery system for liquid laundry detergent compositions which comprises a .beta.-ketoester selected from a Markush group of .beta.-ketoesters. Laundry compositions also contain surfactants and carriers.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (110):

A preferred protease enzyme for use in the present invention is a bleach stable variant of Protease A (BPN'). This bleach stable variant of BPN' is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This bleach stable variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Detailed Description Text - DETX (111):

Protease B A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (131):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especialy the **Bacillus** a-amylases, regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases

vs. the above- identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERIAMYL.RTM., or the homologous position variation of a similar parent amnylase, such as B. amyloliquefaciens, B. subtilis, or B.stearothermophilus; (b) stability-nhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B. licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6184002

DOCUMENT-IDENTIFIER: US 6184002 B1

TITLE: Method for liquefying starch

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 952225

DATE FILED: January 27, 1998

PCT-DATA:
APPL-NO: PCT/US96/08144
DATE-FILED: May 30, 1996
PUB-NO: WO96/38578
PUB-DATE: Dec 5, 1996
371-DATE: Jan 27, 1998
102(E)-DATE:Jan 27, 1998

US-CL-CURRENT: 435/99, 435/201

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of adding a sodium composition to the starch prior to or simultaneously with liquefying the starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Preferred sodium compositions comprise sodium chloride, sodium bicarbonate, sodium benzoate, sodium sulfate, sodium bisulfite, sodium ascorbate, sodium acetate, sodium nitrate, sodium tartrate, sodium tetraborate, sodium propionate, sodium citrate, sodium succinate, monosodium glutamate, trisodium citrate, sodium phosphate or a mixture thereof.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (14):
In U.S. Pat. No. 5,180,669, liquefaction between a pH of 5.0 to 6.0 was

achieved by the addition of carbonate ion in excess of the amount needed to buffer the solution to the ground starch slurry. Due to an increased pH effect which occurs with addition of carbonate ion, the slurry is generally neutralized by adding a source of hydrogen ion, for example, an inorganic acid such as hydrochloric acid or sulfuric acid. In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (15):

In PCT publication No. 94/18314, a **mutant -amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (29):

"Starch solution" means the water soluble gelatinized starch which results from heating granular starch. Upon heating of the granules to above about 72.degree. C., granular starch dissociates to form an aqueous mixture of loose starch molecules. This mixture comprising, for example, about 75% amylopectin and 25% amylose in yellow dent corn forms a viscous solution in water. In commercial processes to form glucose or fructose, it is the starch solution which is liquefied to form a soluble dextrin solution. "**.alpha.-Amylase**" means an enzymatic activity which cleaves or hydrolyzes the .alpha.(1-4) glycosidic bond, e.g., that in starch, amylopectin or amylose polymers. Suitable **.alpha.-amylases** are the naturally occurring **.alpha.-amylases** as well as recombinant or **mutant** amylases which are useful in liquefaction of starch. Preferred amylases in the present invention are **.alpha.-amylases** derived from **Bacillus, and particularly Bacillus** licheniformis, **Bacillus** amyloliquefaciens or **Bacillus** stearothermophilus.

US-PAT-NO: 6162628

DOCUMENT-IDENTIFIER: US 6162628 A

TITLE: Maltogenic alpha-amylase variants

DATE-ISSUED: December 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cherry; Joel	Davis	CA	N/A	N/A
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Andersen; Carsten	Vaerloese	N/A	N/A	DK
Beier; Lars	Lyngby	N/A	N/A	DK
Frandsen; Torben Peter	Frederiksberg	N/A	N/A	DK

APPL-NO: 09/ 386607

DATE FILED: August 31, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of PCT/DK99/00088 filed Feb. 26, 1999 and claims priority under 35 U.S.C. 119 of Danish application no. 98/00269 filed Feb. 27, 1998 and U.S. provisional application no. 60/077,795 filed Mar. 12, 1998, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	98/00269	February 27, 1998

US-CL-CURRENT: 435/202

ABSTRACT:

The inventors have modified the amino acid sequence of a maltogenic alpha-amylase to obtain variants with improved properties, based on the three-dimensional structure of the maltogenic alpha-amylase Novamyl. The variants have altered physicochemical properties, e.g. an altered pH optimum, improved thermostability, increased specific activity, an altered cleavage pattern or an increased ability to reduce retrogradation of starch or staling of bread.

45 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Brief Summary Text - BSTX (95):
Maltogenic Alpha-Amylase **Variants with Altered Stability**

Brief Summary Text - BSTX (96):
A **variant with improved stability** (typically increased stability) may be obtained by stabilization of calcium binding, substitution with proline, substitution of histidine with amonter amino acid, introduction of an interdomain disulfide bond, removal of a deamidation site, altering a hydrogen bond contact, filling in an internal structural cavity with one or more amino acids with bulkier side groups, introduction of interdomain interactions, altering charge distribution, helix capping, or introduction of a salt bridge.

Brief Summary Text - BSTX (119):
Variants with improved stability of the enzyme can be achieved by improving existing or introducing new interdomain and intradomain contacts. Such improved stability can be achieved by the modifications listed below.

Brief Summary Text - BSTX (142):
Another preferred embodiment of the invention relates to a variant of a parent maltogenic alpha-amylase which has an improved **stability and wherein said variant** comprises a substitution in a position corresponding at least one of the following sets of positions in SEQ ID NO: 1:

Brief Summary Text - BSTX (220):
In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Novamyl-like .alpha.-amylase, wherein the **variant exhibits increased stability** at low pH and at low calcium concentration relative to the parent, the method comprising:

Brief Summary Text - BSTX (257):
3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed

Brief Summary Text - BSTX (270):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a maltogenic **alpha-amylase variant** of the invention,

especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarose gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes, etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Brief Summary Text - BSTX (286):

To screen for **variants wit increased stability,** the filter with bound maltogenic alpha-amylase variants can be pretreated prior to the detection step described above to inactivate **variants that do not have improved stability** relative to the parent maltogenic alpha-amylase. This inactivation step may consist of, but is not limited to, incubation at elevated temperatures in the presence of a buffered solution at any pH from 2 to 12, and/or in a buffer containing another compound known or thought to contribute to altered stability e.g., surfactants, EDTA, EGTA, wheat flour components, or any other relevant additives. Filters so treated for a specified time are then rinsed briefly in deionized water and placed on plates for activity detection as described above. The conditions are chosen such that stabilized variants show increased enzymatic activity relative to the parent after incubation on the detection media.

Brief Summary Text - BSTX (287):

To screen for variants with altered thermostability, filters with bound variants are incubated in buffer at a given pH (e.g., in the range from pH 2-12) at an elevated temperature (e.g., in the range from 50.degree.-110.degree. C.) for a time period (e.g., from 1-20 minutes) to inactivate nearly all of the parent maltogenic alpha-amylase, rinsed in water, then placed directly on a detection plate containing immobilized Cibacron Red labelled amylopectin and incubated until activity is detectable. Similarly, pH dependent stability can be screened for by adjusting the pH of the buffer in the above inactivation step such that the parent maltogenic alpha-amylase is inactivated, thereby allowing detection of only those **variants with increased stability** at the pH in question. To screen for **variants with increased calcium-dependent** stability calcium chelators, such as ethylene glycol-bis(.beta.-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), is added to the inactivation buffer at a concentration such that the parent maltogenic alpha-amylase is inactivated under conditions further defined, such as buffer pH, temperature or a specified length of incubation.

US-PAT-NO: 6159687

DOCUMENT-IDENTIFIER: US 6159687 A

TITLE: Methods for generating recombined polynucleotides

DATE-ISSUED: December 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vind; Jesper	Lyngby	N/A	N/A	DK

APPL-NO: 09/ 040697

DATE FILED: March 18, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish applications 0307/97 filed Mar. 18, 1997, 0434/97 filed Apr. 17, 1997, 0625/97 filed May 30, 1997, and U.S. Provisional applications Ser. No. 60/044,836 filed Apr. 25, 1997 and 60/053,012 filed Jun. 24, 1997 the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0307/97	March 18, 1997
DK	0434/97	April 17, 1997
DK	0625/97	May 30, 1997

US-CL-CURRENT: 435/6, 435/91.2 , 435/91.5

ABSTRACT:

A method for in vitro construction of a library of recombined homologous polynucleotides from a number of different starting DNA templates and primers by induced template shifts during an polynucleotide synthesis is described, whereby

A. extended primers are synthesized by

a) denaturing the DNA templates

b) annealing primers to the templates,

c) extending the said primers by use of a polymerase,

d) stop the synthesis, and

e) separate the extended primers from the templates,

B. a template shift is induced by

a) isolating the extended primers from the templates and repeating steps A.b) to A.e) using the extended primers as both primers and templates, or

b) repeating steps A.b) to A.e),

C. this process is terminated after an appropriate number of cycles of process steps A. and B.a), A. and B.b), or combinations thereof.

Optionally the polynucleotides are amplified in a standard PCR reaction with specific primers to selectively amplify homologous polynucleotides of interest.

126 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (131):
Strauberg et al. (Biotechnology 13: 669-673 (1995) describes a screening system for subtilisin **variants having Calcium-independent stability**;

Detailed Description Text - DETX (99):
In Example 1, it was shown how a number of multiple **variants** of H. lanuginosa lipase were shuffled. In a similar manner, **variants of Bacillus .alpha.-amylases** can be shuffled.

Detailed Description Text - DETX (100):
Earlier patent applications have identified **variants of various .alpha.-amylases from Bacillus** species improved for particular properties, e.g. thermostability, stability under Calcium-depleted conditions, improved wash-performance etc. (see WO95/10603, WO96/23874, WO96/23873, and PCT/DK97/00197).

US-PAT-NO: 6143708

DOCUMENT-IDENTIFIER: US 6143708 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Borchert; Torben Vedel	Jyllinge	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd	N/A	N/A	DK

APPL-NO: 09/ 182859

DATE FILED: October 29, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0515/96	April 30, 1996
DK	0712/96	June 28, 1996
DK	0775/96	July 11, 1996
DK	1263/96	November 8, 1996

US-CL-CURRENT: 510/226, 435/202 , 435/252.3 , 435/320.1 , 510/326 , 510/392 , 536/23.2 , 536/23.7

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like .alpha.-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

92 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Among more recent disclosures relating to **.alpha.-amylases,** WO 96/23874 provides three-dimensional, X-ray crystal structural data for a Termamyl-like **.alpha.-amylase** which consists of the 300 N-terminal amino acid residues of the B. amyloliquefaciens **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 4 herein and amino acids 301-483 of the C-terminal end of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 2 herein (the latter being available commercially under the tradename Termamyl.TM.), and which is thus closely related to the industrially important **Bacillus .alpha.-amylases** (which in the present context are embraced within the meaning of the term "Termamyl-like **.alpha.-amylases**", and which include, inter alia, the B. licheniformis, B. amyloliquefaciens and B. stearothermophilus **.alpha.-amylases**). WO 96/23874 further describes methodology for designing, on the basis of an analysis of the structure of a parent Termamyl-like **.alpha.-amylase, variants** of the parent Termamyl-like **.alpha.-amylase** which exhibit altered properties relative to the parent.

Brief Summary Text - BSTX (180):

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent Termamyl-like .alpha.-amylase, which **variant exhibits increased stability** at low pH and at low calcium concentration relative to the parent, the method comprising:

Brief Summary Text - BSTX (208):

.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Brief Summary Text - BSTX (215):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA

sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaceins **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Brief Summary Text - BSTX (319):

3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired **stability and/or performance of the variant** to be constructed

Detailed Description Text - DETX (60):

Construction, by Localized Random, Doped Mutagenesis, of Termamyl-like .alpha.-amylase **Variants Having an Improved Stability** at Low pH and a Reduced Dependency on Calcium Ions for Stability Compared to the Parent Enzyme

Detailed Description Text - DETX (199):

The mutations indicated in bold were introduced by the random mutagenesis method. The **stability data for these variants** appear from Table 11 in Example 3.

Detailed Description Text - DETX (222):

This example summarises the **stability results of variants** characterised by a fluorimetric assay at 70.degree. C. under two different conditions, (1) pH 4.5 and 1 mM CaCl.sub.2 and (2) pH 6.2 and 10 .mu.M CaCl.sub.2.

Claims Text - CLTX (46):

23. A method for producing a variant of a parent Termamyl-like .alpha.-amylase of claim 1, which **variant exhibits increased stability** at low pH and at low calcium concentration relative to the parent, the method comprising:

US-PAT-NO: 6121226

DOCUMENT-IDENTIFIER: US 6121226 A
See image for Certificate of Correction

TITLE: Compositions comprising cotton soil release polymers and protease enzymes

DATE-ISSUED: September 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gosselink; Eugene Paul	Cincinnati	OH	N/A	N/A
Price; Kenneth Nathan	Wyoming	OH	N/A	N/A
Ghosh; Chanchal Kumar	West Chester	OH	N/A	N/A

APPL-NO: 09/ 354972

DATE FILED: July 16, 1999

PARENT-CASE:

CROSS REFERENCE

This application is a continuation in part of Ser. No. 09/180,191 filed Nov. 3, 1998 now U.S. Pat. No. 6,087,316, and a provision of Ser. No. 60/016,807 filed May 3, 1996, which is a continuation-in-part of Ser. No. PCT/US97/06917 filed Apr. 25, 1997.

US-CL-CURRENT: 510/400, 510/299 , 510/300 , 510/305 , 510/306 , 510/405 , 510/499 , 510/504 , 510/517 , 510/528 , 510/530

ABSTRACT:

Cotton soil release polymers comprising water soluble and/or dispersible, modified polyamines having functionalized backbone moieties and improved stability toward bleach. Also, laundry detergent compositions comprising these cotton soil release polymers having enhanced hydrophilic soil removal benefits in combination with a protease enzyme system are provided.

24 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (33):
A preferred protease enzyme for use in the present invention is a variant of

Protease A (BPN') which is a non-naturally occurring carbonyl hydrolase **variant having a different pr te lytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to

Detailed Description Text - DETX (36):

A preferred protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occurring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Detailed Description Text - DETX (85):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB. 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, June 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 60.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B.licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stearothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17, 1994, by C. Mitchinson. Therein it was noted that

bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B.licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 6093562

DOCUMENT-IDENTIFIER: US 6093562 A

TITLE: Amylase variants

DATE-ISSUED: July 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen N	N/A	N/A	DK

APPL-NO: 08/ 600656

DATE FILED: February 13, 1996

PARENT-CASE:

This application is a continuation of international application PCT/DK96/00056, filed Feb. 5, 1996.

US-CL-CURRENT: 435/202, 510/226 , 510/236 , 510/320 , 510/392 , 510/530

ABSTRACT:

The present invention relates to variants of a parent .alpha.-amylase, which parent .alpha.-amylase (i) has an amino acid sequence selected from the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, and SEQ ID No. 7, respectively; or (ii) displays at least 80% homology with one or more of these amino acid sequences; and/or displays immunological cross-reactivity with an antibody raised against an .alpha.-amylase having one of these amino acid sequences; and/or is encoded by a DNA sequence which hybridizes with the same probe as a DNA sequence encoding an .alpha.-amylase having one of these amino acid sequences; in which variant:

(a) at least one amino acid residue of the parent .alpha.-amylase has been deleted; and/or

(b) at least one amino acid residue of the parent .alpha.-amylase has been replaced by a different amino acid residue; and/or

(c) at least one amino acid residue has been inserted relative to the parent .alpha.-amylase; the variant having .alpha.-amylase activity and exhibiting at least one of the following properties relative to the parent .alpha.-amylase: increased thermostability; increased stability towards oxidation; and reduced Ca.sup.2+ dependency;

with the proviso that the amino acid sequence of the variant is not

identical to any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively.

5 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Brief Summary Text - BSTX (9):
WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Brief Summary Text - BSTX (14):
EP525 610 relates to **mutant enzymes having improved stability** towards ionic tensides (surfactants). The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations are not specified.

Brief Summary Text - BSTX (15):
WO 94/02597 discloses .alpha.-amylase **mutants which exhibit improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Brief Summary Text - BSTX (18):
An object of the present invention is to provide .alpha.-amylase variants which--relative to their parent .alpha.-amylase--possess improved properties of importance, inter alia, in relation to the washing and/or dishwashing performance of the **variants in question, e.g. increased thermal stability,** increased stability towards oxidation, reduced dependency on Ca.sup.2+ ion and/or improved stability or activity in the pH region of relevance in, e.g., laundry washing or dishwashing. Such variant .alpha.-amylases have the advantage, among others, that they may be employed in a lower dosage than their

parent .alpha.-amylase. Furthermore, the .alpha.-amylase variants may be able to remove starchy stains which cannot, or can only with difficulty, be removed by .alpha.-amylase detergent enzymes known today.

Brief Summary Text - BSTX (94):

From the results obtained by the present inventors it appears that changes in a particular property, e.g. thermal **stability or oxidation stability, exhibited by a variant** relative to the parent .alpha.-amylase in question can to a considerable extent be correlated with the type of, and positioning of, mutation(s) (amino acid substitutions, deletions or insertions) in the variant. It is to be understood, however, that the observation that a particular mutation or pattern of mutations leads to changes in a given property in no way excludes the possibility that the mutation(s) in question can also influence other properties.

Brief Summary Text - BSTX (95):

Oxidation stability: With respect to increasing the oxidation **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable that at least one, and preferably multiple, oxidizable amino acid residue(s) of the parent has/have been deleted or replaced (i.e. substituted by) a different amino acid residue which is less susceptible to oxidation than the original oxidizable amino acid residue.

Brief Summary Text - BSTX (96):

Particularly relevant oxidizable amino acid residues in this connection are cysteine, methionine, tryptophan and tyrosine. Thus, for example, in the case of parent .alpha.-amylases containing cysteine it is anticipated that deletion of cysteine residues, or substitution thereof by less oxidizable amino acid residues, will be of importance in obtaining **variants with improved oxidation stability** relative to the parent .alpha.-amylase.

Brief Summary Text - BSTX (97):

In the case of the above-mentioned parent .alpha.-amylases having the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, respectively, all of which contain no cysteine residues but have a significant methionine content, the deletion or substitution of methionine residues is particularly relevant with respect to achieving improved oxidation **stability of the resulting variants**. Thus, deletion or substitution [e.g. by threonine (T), or by one of the other amino acids listed above] of one or more of the methionine residues in positions M9, M10, M105, M202, M208, M261, M309, M382, M430 and M440 of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7, and/or in position M323 of the amino acid sequence shown in SEQ ID No. 2 (or deletion or substitution of methionine residues in equivalent positions in the sequence of another .alpha.-amylase meeting one of the other criteria for a parent .alpha.-amylase mentioned above) appear to be particularly effective with respect to increasing the oxidation stability.

Brief Summary Text - BSTX (106):

Thermal stability: With respect to increasing the thermal **stability of an .alpha.-amylase variant** relative to its parent .alpha.-amylase, it appears to be particularly desirable to delete at least one, and preferably two or even three, of the following amino acid residues in the amino acid sequence shown in SEQ ID No. 1 (or their equivalents): F180, R181, G182, T183, G184 and K185. The corresponding, particularly relevant (and equivalent) amino acid residues in the amino acid sequences shown in SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 7, respectively, are: F180, R181, G182, D183, G184 and K185 (SEQ ID No. 2); F178, R179, G180, I 181, G182 and K183 (SEQ ID No. 3); and F180, R181, G182, H183, G184 and K185 (SEQ ID No. 7).

Brief Summary Text - BSTX (114):

Examples of specific mutations which appear to be of importance in connection with the thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequence shown in SEQ ID No. 1 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): K269R; P260E, R124P, M105F,I,L,V; M208F,W,Y; L217I; V206I,L,F.

Brief Summary Text - BSTX (118):

Still further examples of mutations which appear to be of importance, inter alia, in achieving improved thermal **stability of an .alpha.-amylase variant** relative to the parent .alpha.-amylase in question are one or more of the following substitutions in the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 7 (or equivalents thereof in another parent .alpha.-amylase in the context of the invention): A354C+V479C; L351C+M430C; N457D,E+K385R; L355D,E+M430R,K; L355D,E+I411R,K; and N457D,E.

Brief Summary Text - BSTX (213):

.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No.1 can barely be detected. Stabilized variants show, under the same conditions, increased color intensity due to increased liberation of Cibacron Red.

Brief Summary Text - BSTX (223):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces

coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xy1A and xy1B genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (75):
Determination of Oxidation **Stability of M202 Substitution Variants** of the Parent .alpha.-amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detailed Description Text - DETX (76):
A: Oxidation **Stability of Variants** of the Sequence in SEQ ID No. 1

Detailed Description Text - DETX (81):
B: Oxidation **Stability of Variants** of the Sequence in SEQ ID No. 2

Detailed Description Text - DETX (85):
Determination of Thermal **Stability of Variants** of the Parent .alpha.-amylases Having the Amino Acid Sequences Shown in SEQ ID No. 1 and SEQ ID No. 2

Detailed Description Text - DETX (86):
A: Thermal **Stability of Pairwise Deletion Variants** of the Sequence in SEQ ID No. 1

Detailed Description Text - DETX (94):
It is apparent that all of the pairwise deletion variants tested exhibit significantly improved thermal stability relative to the parent .alpha.-amylase (SEQ ID No. 1), and that the thermal **stability of Variant** 5, which in addition to the pairwise deletion mutation of Variant 4 comprises the substitution R124P, is markedly higher than that of the other variants. Since calorimetric results for the substitution variant R124P (comprising only the substitution R124P) reveal an approximately 7.degree. C. thermostabilization thereof relative to the parent .alpha.-amylase, it appears that the thermostabilizing effects of the mutation R124P and the pairwise deletion, respectively, reinforce each other.

Detailed Description Text - DETX (95):
B: Thermal **Stability of Pairwise Deleti n Variants** of the Sequence in SEQ ID

No. 2

Detailed Description Text - DETX (101):
C: Thermal **Stability f a Multi-combinati n Variant** of the Sequence in SEQ ID No. 1

US-PAT-NO: 6080568

DOCUMENT-IDENTIFIER: US 6080568 A

TITLE: **Mutant .alpha.-amylase** comprising modification at residues corresponding to A210, H405 and/or T412 in **Bacillus** licheniformis

DATE-ISSUED: June 27, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Day; Anthony G.	San Francisco	CA	N/A	N/A
Swanson; Barbara A.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 914679

DATE FILED: August 19, 1997

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/275 , 435/440 , 435/832 , 435/836 , 510/320 , 570/226 , 570/235

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more of residues corresponding to A210, H405 and T412 in Bacillus licheniformis are mutated. The disclosed .alpha.-amylase enzymes show altered or improved stability and/or activity profiles.

11 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

TITLE - TI (1):
Mutant .alpha.-amylase comprising modification at residues corresponding to A210, H405 and/or T412 in **Bacillus** licheniformis

Brief Summary Text - BSTX (2):
The present invention is directed to .alpha.-amylases having introduced therein mutations providing additional stability under certain conditions. It is specifically contemplated that the **mutant will have altered perf rmance characteristics such as altered stability** and/or altered activity profiles.

Brief Summary Text - BSTX (16):
In PCT Publication No. WO 95/35382, a **mutant .alpha.-amylase is described having improved oxidati n stability** and having changes at positions 104, 128, 187 and/or 188 in B. licheniformis .alpha.-amylase.

Brief Summary Text - BSTX (18):
In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (19):
In PCT publication No. WO 94/18314, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Detailed Description Text - DETX (5):
"Host strain" or "host cell" means a suitable host for an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (.DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (20):
Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Re. 34,606.

Detailed Description Text - DETX (22):
A wide variety of host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and

prokaryotic hosts, such as strains of E. coli, Pseudomonas, **Bacillus,** Streptomyces, various fungi, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (44):
Transformation of Plasmids Into **Bacillus** subtilis, Expression and Purification of **Mutant .alpha.-Amylase**

Detailed Description Text - DETX (45):
.alpha.-Amylase may be expressed in **Bacillus** subtilis after transformation with the plasmids described above. pHP13 is a plasmid able to replicate in E. coli and in **Bacillus** subtilis. Plasmids containing different **variants** were constructed using E. coli strain MM294, the plasmids isolated and then transformed into **Bacillus** subtilis as described in Anagnostopoulos et al., J. Bacter., Vol. 81, pp. 741-746 (1961). The **Bacillus** strain had been deleted for two proteases (.DELTA.apr, .DELTA.npr) (see e.g., Ferrari et al., U.S. Pat. No. 5,264,366) and for amylase (.DELTA.amyE) (see e.g., Stahl et al., J. Bacter., Vol. 158, pp. 411-418 (1984)). After transformation, the sacU(Hy) mutation (Henner et al., J. Bacter., Vol., 170, pp. 296-300 (1988)) was introduced by PBS-1 mediated transduction (Hoch, J. Bact., Vol. 154, pp. 1513-1515 (1983)).

Detailed Description Text - DETX (53):
Preparation and Testing of Additional **Mutant Alpha-Amylases for Thermal Stability**

Claims Text - CLTX (1):
1. A **mutant .alpha.-amylase** which is derived from a precursor **.alpha.-amylase** by the substitution to said precursor **.alpha.-amylase** of a residue corresponding to T412 in **Bacillus** licheniformis **.alpha.-amylase** as numbered in SEQ. ID NO:3.

Other Reference Publication - OREF (53):
Mizuno et al., "Crystallization and Preliminary X-ray Studies of Wild Type and Catalaytic-site **Mutant .alpha.-Amylase from bacillus** subtilis," J Mol Biol (1993) 234:1282-1283.

US-PAT-NO: 6022724

DOCUMENT-IDENTIFIER: US 6022724 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: February 8, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben	Copenhagen N	N/A	N/A	DK

APPL-NO: 08/ 683838

DATE FILED: July 18, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Ser. No. 08/600,908 filed Feb. 13, 1996 which is a 371 of PCT/DK96/00057 filed Feb. 5, 1996, which are incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0128/95	February 3, 1995
DK	1192/95	October 23, 1995
DK	1256/95	November 10, 1995

US-CL-CURRENT: 435/202, 435/203 , 510/226 , 510/235 , 510/320 , 510/392

ABSTRACT:

The present invention relates to a method of constructing a variant of a parent Termamyl-like .alpha.-amylase, which variant has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprises

i) analyzing the structure of the parent Termamyl-like .alpha.-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like .alpha.-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent Termamyl-like .alpha.-amylase (as evaluated on the basis of structural or functional considerations),

ii) constructing a Termamyl-like .alpha.-amylase variant, which as compared to the parent Termamyl-like .alpha.-amylase, has been modified in the amino

acid residue or structural part identified in i) so as to alter the property, and, optionally,

iii) testing the resulting Termamyl-like .alpha.-amylase variant with respect to the property in question.

5 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

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Detailed Description Text - DETX (337):
.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID NO: 2 can barely be detected. Stabilized variants show, under the same conditions, increased colour intensity due to increased liberation of Cibacron Red.

Detailed Description Text - DETX (344):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

US-PAT-NO: 6008026

DOCUMENT-IDENTIFIER: US 6008026 A

TITLE: Mutant .alpha.-amylase having introduced therein a disulfide bond

DATE-ISSUED: December 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Day; Anthony G.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 890383

DATE FILED: July 11, 1997

US-CL-CURRENT: 435/96, 435/202 , 435/203 , 435/204 , 435/205 , 435/262 , 435/267 , 435/274 , 435/275 , 435/320.1 , 536/23.2

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more disulfide bonds are introduced into the enzyme via addition or substitution of a residue with a cysteine. The disclosed .alpha.-amylase enzymes show altered or improved stability and/or activity profiles.

15 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Brief Summary Text - BSTX (2):

The present invention is directed to mutant .alpha.-amylases having introduced therein one or more disulfide bonds. In particular, the disulfide bonds are introduce by mutation of a precursor .alpha.-amylase to introduce one or more cysteine residues so as to produce a disulfide bond between two cysteine residues in said mutant .alpha.-amylase. It is specifically contemplated that the **mutant will have altered performance characteristics such as altered stability** and/or altered activity profiles.

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 95/35382, a **mutant .alpha.-amylase is described having improved oxidation stability** and having changes at positions 104, 128,

187 and/or 188 in B. licheniformis .alpha.-amylase.

Brief Summary Text - BSTX (18):
In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having impr ved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (19):
In PCT publication No. WO 94/18314, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (27):
Accordingly, the present invention provides a **mutant .alpha.-amylase** having introduced therein one or more cysteine residues, wherein at least one of the introduced cysteine residues is capable of forming a disulfide bond with another cysteine residue. Preferably, the introduced cysteine(s) and the other cysteine residue with which it is to form a disulfide bond correspond to positions in the precursor **.alpha.-amylase** having a C.alpha.-C.alpha. bond distance of between about 4.4-6.8 Angstroms and a C.beta.-C.beta. bond distance of between about 3.45 and 4.5 Angstroms. In a particularly preferred embodiment of the invention, the **.alpha.-amylase** is derived from a bacterial or a fungal source and comprises a substitution corresponding to E119C/S130C and/or D124C/R127C **Bacillus** licheniformis. Most preferably, the **.alpha.-amylase** is derived from **Bacillus**.

Detailed Description Text - DETX (5):
"Host strain" or "host cell" means a suitable host for an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (.DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (7):
According to the present invention, a **mutant .alpha.-amylase** is provided that has introduced therein a first cysteine residue which is capable of forming a disulfide bond with a second cysteine residue. Preferably, the first

cysteine residue comprises an addition or a substitution to a precursor **.alpha.-amylase**. It is further possible to incorporate the second cysteine residue as an addition or substitution as well, and this may be preferable should a useful cysteine residue not be present in a location useful to stabilize the desired portion of the molecule. With respect to **Bacillus** licheniformis **.alpha.-amylase,** it is necessary to incorporate two cysteine residues as the wild type molecule possesses no cysteines. Addition or substitution of an amino acid as used herein refers to any modification of the amino acid sequence itself of the precursor **.alpha.-amylase,** but preferably refers to using genetic engineering to mutate a nucleic acid encoding the precursor **.alpha.-amylase** so as to encode the substituted or added cysteine residue in the expressed protein. The precursor **.alpha.-amylases** include naturally occurring **.alpha.-amylases** and recombinant **.alpha.-amylases**. Modification of the precursor DNA sequence which encodes the amino acid sequence of the precursor **.alpha.-amylase** can be by methods described herein and in commonly owned U.S. Pat. Nos. 4,760,025 and 5,185,258, incorporated herein by reference.

Detailed Description Text - DETX (21):

Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Re. 34,606.

Detailed Description Text - DETX (23):

A wide variety of host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well-known eukaryotic and prokaryotic hosts, such as strains of E. coli, Pseudomonas, **Bacillus,** Streptomyces, various fungi, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (24):

The improved **.alpha.-amylases** according to the present invention are contemplated to provide several important advantages when compared to wild type **Bacillus .alpha.-amylases**. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and oxidative stability which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is

that the addition of equal protein doses of **.alpha.-amylase** according to the invention provide superior performance when compared to wild type **Bacillus** licheniformis **.alpha.-amylase** due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this **variant**. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

Detailed Description Text - DETX (49):
Transformation of Plasmids Into **Bacillus** subtilis, Expression And Purification of **Mutant .alpha.-Amylase**

Detailed Description Text - DETX (50):
.alpha.-Amylase may be expressed in **Bacillus** subtilis after transformation with the plasmids described above. pHP13 is a plasmid able to replicate in E. coli and in **Bacillus** subtilis. Plasmids containing different **variants** were constructed using E. coli strain MM294, the plasmids isolated and then transformed into **Bacillus** subtilis as described in Anagnostopoulos et al., J. Bacter., Vol. 81, pp. 741-746 (1961). The **Bacillus** strain had been deleted for two proteases (.DELTA.apr, .DELTA.npr) (see e.g., Ferrari et al., U.S. Pat. No. 5,264,366) and for amylase (.DELTA.amyE) (see e.g., Stahl et al., J. Bacter., Vol. 158, pp. 411-418 (1984)). After transformation, the sacU(Hy) mutation (Henner et al., J. Bacter., Vol., 170, pp. 296-300 (1988)) was introduced by PBS-1 mediated transduction (Hoch, J. Bact., Vol. 154, pp. 1513-1515 (1983)).

Detailed Description Text - DETX (58):
Preparation and Testing of Additional **Mutant Alpha-Amylases for Thermal Stability**

Detailed Description Text - DETX (60):
As shown in Table 1, mutant enzymes having introduced therein two cysteine residues capable of forming a disulfide bond showed significantly increased **stability over the mutant** M15T enzyme with no introduces cysteine bonds. Additionally, as shown in Table 1, mutant enzymes having introduced therein a disulfide bond between E119C and S130C showed significantly improved **stability over the M15T mutant** or the M15T/E119C/S130C mutant which was treated with DTT (i.e., disulfide bond reduced and/or broken).

US-PAT-NO: 6004790

DOCUMENT-IDENTIFIER: US 6004790 A
See image for Certificate of Correction

TITLE: Cyclomaltodextrin glucanotransferase variants

DATE-ISSUED: December 21, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dijkhuizen; Lubbert	Groningen	N/A	N/A	NL
Dijkstra; Bauke W.	Groningen	N/A	N/A	NL
Andersen; Carsten	Bagsvaerd	N/A	N/A	DK
Osten; Claus von der	Bagsvaerd	N/A	N/A	DK

APPL-NO: 08/ 947965

DATE FILED: October 9, 1997

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. PCT/DK96/00179 filed Apr. 22, 1996 and claims priority under 35 U.S.C. 119 of Danish application Ser. Nos. 0477/95, 1173/95 and 1281/95 filed Apr. 21, 1995, Oct. 17, 1995 and Nov. 16, 1995, respectively, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0477/95	April 21, 1995
DK	1173/95	October 17, 1995
DK	1281/95	November 16, 1995

US-CL-CURRENT: 435/193, 435/320.1 , 536/23.2

ABSTRACT:

The present invention relates to variants of cyclomaltodextrin glucanotransferase. More specifically the invention relates to a method of modifying the substrate binding and/or product selectivity of a precursor CGTase enzyme, and CGTase variants derived from a precursor CGTase enzyme by substitution, insertion and/or deletion of one or more amino acid residue(s), which amino acid residue(s) holds a position close to the substrate. Moreover, the invention relates to DNA constructs encoding the CGTase variants, expression vectors, host cells and methods of producing the CGTase variants of the invention.

62 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Detailed Description Text - DETX (317):
Examples of suitable promoters for directing the transcription of the DNA encoding the CGTase **variant** of the invention in bacterial host cells include the promoter of the **Bacillus** stearothermophilus maltogenic amylase gene, the **Bacillus** licheniformis **alpha-amylase** gene, the **Bacillus** amyloliquefaciens BAN amylase gene, the **Bacillus** subtilis alkaline protease gen, or the **Bacillus** pumilus xylanase or xylosidase gene, or by the phage Lambda P.sub.R or P.sub.L promoters or the E. coli lac, trp or tac promoters.

Detailed Description Text - DETX (373):
Bacterial Strains and Plasmids: Escherichia coli MC1061 [Meissner P S, Sisk W P, Berman M L; Proc. Natl. Acad. Sci. USA 1987 84 4171-4175] was used for recombinant DNA manipulations and site-directed mutagenesis. E. coli DH5.alpha. [Hanahan D; J. Mol Biol. 1983 166 557] was used for the production of monomeric supercoiled plasmid DNA for sequencing. CGTases **variants** were produced with the **.alpha.-amylase** and protease negative **Bacillus** subtilis Strain DB104A [Smith H, de Jong A, Bron S, Venema G; Gene 1988 70 351-361]. The fragment containing the kanamycin-resistance marker was ligated with the largest fragment from plasmid pDP66S [Penninga D, Strokopytov B, Rozeboom H J, Lawson C L, Dijkstra B W, Bergsma J, Dijkhuizen L; Biochemistry 1995 34 3368-3376] containing the **Bacillus** circulans CGTase gene, digested with HIndIII and Xbal (made blunt with Klenow polymerase). The resulting CGTase protein expression shuttle vector pDP66K, with the CGTase gene under control of the erthromycin-inducible p32 promotor [van der Vossen J M B M, Kodde J, Haandrikman A J, Venema G, Kok J; Appl. Environ. Microbiol. 1992 58 3142-3149], was transformed to E. coli MC1061 under selection for erythromycin and kanamycin resistance, cf. FIG. 3.

Detailed Description Text - DETX (374):
Construction of CGTase **Variants: As only relatively low stability** with plasmid pDP66S (8.5 kb) [Saenger W; Angew. Chem. 1980 19 344-362] was found, pDP66K (7.7 kb) was constructed, cf. FIG. 3, with the CGTase gene under the control of the strong p32 promotor [van der Vossen J M B M, Kodde J, Haandrikman A J, Venema G, Kok J; Appl. Environ. Microbiol. 1992 58 3142-3149]. Plasmid pDP66K containing the additional antibiotic resistance marker for kanamycin appeared to be considerably more stable in E. coli as well as in B. subtilis cells than plasmid pDP66S containing the streptomycin/spectinomycin resistance cassette. Using this shuttle vector, a high extracellular production of wild-type enzyme and CGTase variants was obtained reproducibly in batch fermentations with the .alpha.-amylase and protease negative B. subtilis Strain DB104A. A single 51 erlenmeyer flask with

11 B. subtilis Strain DB104A culture allowed purification to homogeneity of up to 25 mg of the CGTase variants. Mutations were constructed via site-directed (PCR) mutagenesis. Using specific oligonucleotide primers a mutation frequency close to 70% was observed. All mutations were confirmed by restriction analysis and DNA sequencing.

Detailed Description Text - DETX (419):

Mutations were introduced by a method based on PCR by the use of PWO polymerase. For each mutation, specific oligonucleotides (primers) were developed. The mutations were confirmed by restriction analysis whenever possible, and by sequencing. **Mutant** proteins were expressed in either Escherichia coli MC1061 [Meissner P S, Sisk W P, Berman M L; Proc. Natl. Acad. Sci. USA 1987 84 4171-4175], or in the **.alpha.-amylase** and protease negative **Bacillus** subtilis Strain DB104A [Smith H, de Jong A, Bron S, Venema G; Gene 1988 70 351-361]. Proteins were purified from the media using affinity chromatography (AfC) and/or anion-exchange chromatography (AEC).

Detailed Description Text - DETX (474):

Mutations were introduced by a method based on PCR by the use of PWO polymerase. For each mutation, specific oligonucleotides (primers) were developed. The mutations were confirmed by restriction analysis whenever possible, and by sequencing. **Mutant** proteins were expressed in either Escherichia coli MC1061 [Meissner P S, Sisk W P, Berman M L; Proc. Natl. Acad. Sci. USA 1987 84 4171-4175], or in the **.alpha.-amylase** and protease negative **Bacillus** subtilis Strain DB104A [Smith H, de Jong A, Bron S, Venema G; Gene 1988 70 351-361]. Proteins were purified from the media using affinity chromatography (AfC) and/or anion-exchange chromatography (AEC).

US-PAT-NO: 5989169

DOCUMENT-IDENTIFIER: US 5989169 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkeroed	N/A	N/A	DK
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben Vedel	Copenhagen N	N/A	N/A	DK

APPL-NO: 08/ 600908

DATE FILED: February 13, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK96/00057 filed Feb. 5, 1996, which is incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0128/95	February 3, 1995
DK	1192/95	October 23, 1995
DK	1256/95	November 10, 1995

US-CL-CURRENT: 435/201, 435/202 , 435/203 , 435/204 , 435/252.3 , 435/440 , 536/23.2

ABSTRACT:

The present invention relates to a method of constructing a variant of a parent Termamyl-like .alpha.-amylase, which variant has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprising i) analyzing the structure of the parent Termamyl-like .alpha.-amylase to identify at least one amino acid residue or at least one structural part of the Termamyl-like .alpha.-amylase structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent Termamyl-like .alpha.-amylase (as evaluated on the basis of structural or functional considerations), ii) constructing a Termamyl-like .alpha.-amylase variant, which as compared to the parent Termamyl-like .alpha.-amylase, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and iii) testing the resulting Termamyl-like .alpha.-amylase variant for the property in question.

23 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

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Detailed Description Text - DETX (285):
.alpha.-Amylase activity is detected by Cibacron Red labelled amylopectin, which is immobilized on agarose. For screening for **variants with increased thermal and high-pH stability, the filter with bound .alpha.-amylase variants** is incubated in a buffer at pH 10.5 and 60.degree. or 65.degree. C. for a specified time, rinsed briefly in deionized water and placed on the amylopectin-agarose matrix for activity detection. Residual activity is seen as lysis of Cibacron Red by amylopectin degradation. The conditions are chosen to be such that activity due to the .alpha.-amylase having the amino acid sequence shown in SEQ ID No. 2 can barely be detected. Stabilized variants show, under the same conditions, increased color intensity due to increased liberation of Cibacron Red.

Detailed Description Text - DETX (293):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

US-PAT-NO: 5958739

DOCUMENT-IDENTIFIER: US 5958739 A

TITLE: Mutant .alpha.-amylase

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Palo Alto	CA	N/A	N/A
Requadt; Carol	Palo Alto	CA	N/A	N/A
Ropp; Traci	Palo Alto	CA	N/A	N/A
Solheim; Leif P.	Palo Alto	CA	N/A	N/A
Ringer; Christopher	Palo Alto	CA	N/A	N/A
Day; Anthony	Palo Alto	CA	N/A	N/A

APPL-NO: 08/ 704706

DATE FILED: February 20, 1997

PCT-DATA:
APPL-NO: PCT/US96/09089
DATE-FILED: June 6, 1996
PUB-NO: WO96/39528
PUB-DATE: Dec 19, 1996
371-DATE: Feb 20, 1997
102(E)-DATE:Feb 20, 1997

US-CL-CURRENT: 435/99, 435/201 , 435/202 , 435/203 , 435/204 , 435/252.3 , 435/252.31 , 435/254.11 , 435/320.1 , 435/325 , 435/410 , 510/226 , 510/300 , 510/305 , 510/320 , 510/374 , 510/392 , 536/23.2

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more asparagine residues are substituted with a different amino acid or deleted. The disclosed .alpha.-amylase enzymes show altered or improved low pH starch hydrolysis performance, stability and activity profiles.

32 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (16):
In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (17):
In PCT publication No. WO 94/18314, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Drawing Description Text - DRTX (10):
FIG. 9 illustrates a schematic of the PCR method used to produce the **mutant** oligonucleotides corresponding to **.alpha.-amylase** derived from **Bacillus** licheniformis.

Drawing Description Text - DRTX (11):
FIG. 10 illustrates a graph derived from a statistical analysis of **variant** enzyme according to the invention, M15T/N188S, compared to wild type **Bacillus** licheniformis **.alpha.-amylase** in starch liquefaction at 107.degree. C., 60 ppm calcium and varying pH.

Drawing Description Text - DRTX (12):
FIG. 11 illustrates a graph derived from a statistical analysis of the performance of a **variant** enzyme according to the invention, M15T/N188S, compared to wild type **Bacillus** licheniformis **.alpha.-amylase** in starch liquefaction at 107.degree. C., pH 6.0 and varying calcium concentration.

Drawing Description Text - DRTX (13):
FIG. 12 illustrates a graph derived from a statistical analysis of the performance of a **variant** enzyme according to the invention, M15T/N188S, compared to wild type **Bacillus** licheniformis **.alpha.-amylase** in starch liquefaction at pH 6.0, 60 ppm calcium and varying temperature.

Detailed Description Text - DETX (5):
"Host strain" or "host cell" means a suitable host for an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (.DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected

with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (19):

Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Re. Pat. No. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Re. Pat. No. 34,606.

Detailed Description Text - DETX (21):

A wide variety of host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of E. coli, Pseudomonas, **Bacillus,** Streptomyces, various fungi, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (22):

The improved **.alpha.-amylases** according to the present invention provide several important advantages when compared to wild type **Bacillus .alpha.-amylases**. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and oxidative stability which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of **.alpha.-amylase** according to the invention provide superior performance when compared to wild type **Bacillus** licheniformis **.alpha.-amylase** due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this **variant**. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

Detailed Description Text - DETX (51):
Transformation of Plasmids into **Bacillus** subtilis, Expression and Purification of **Mutant .alpha.-Amylase**

Detailed Description Text - DETX (52):
.alpha.-Amylase was expressed in **Bacillus** subtilis after transformation with the plasmids described in Examples 1-3. pHP13 is a plasmid able to replicate in E. coli and in **Bacillus** subtilis. Plasmids containing different **variants** were constructed using E. coli strain MM294, the plasmids isolated and then transformed into **Bacillus** subtilis as described in Anagnostopoulos et al., J. Bacter., vol. 81, pp. 741-746 (1961). The **Bacillus** strain had been deleted for two proteases (.DELTA.apr, .DELTA.npr) (see e.g., Ferrari et al., U.S. Pat. No. 5,264,366) and for amylase (.DELTA.amyE) (see e.g., Stahl et al., J. Bacter., vol. 158, pp. 411-418 (1984)). The **bacillus** strain expressing M15L/N188Y was found to form larger zones of clearing than the strain expressing M15L on agar plates containing 1% insoluble starch indicating increased amylolytic activity. After transformation, the sacU(Hy) mutation (Henner et al., J. Bacter., vol., 170, pp. 296-300 (1988)) was introduced by PBS-1 mediated transduction (Hoch, J. Bact., vol. 154, pp. 1513-1515 (1983)).

Detailed Description Text - DETX (81):
.alpha.-Amylase comprising the substitution M15T/N188S made as per Examples 1-4 was compared with wild type **.alpha.-amylase** derived from **Bacillus** licheniformis (Spezyme.RTM. AA20, available commercially from Genencor International, Inc.) in liquefaction at 105.5.degree. C. As shown in Table 2, the **mutant** enzymes provided significantly increased performance in jet-liquefaction of starch, especially at low pH. Pilot scale liquefaction was performed with a primary stage liquefaction at 105.5.degree. C. and a secondary stage liquefaction at 95.degree. C. Amylase was added at 12 LU/g of carbohydrate (dry basis).

Detailed Description Text - DETX (84):
.alpha.-Amylase comprising substitution M15T/N188S made as per Examples 1-4 was compared with wild type **.alpha.-amylase** derived from **Bacillus** licheniformis (Spezyme.RTM. AA20, available commercially from Genencor International, Inc.) in liquefaction at 107.degree. C. As shown in Table 3, the **mutant** enzymes provided significantly increased performance in jet-liquefaction of starch especially at low pH, as shown by the DE value, during liquefaction processes. Pilot scale liquefaction was performed with a primary stage liquefaction at 107.degree. C. and a secondary stage liquefaction at 95.degree. C. Amylase was added at 12 LU/g or carbohydrate (dry basis).

Detailed Description Text - DETX (90):
Preparation and Testing of Additional **Mutant Alpha-Amylases for Thermal Stability**

Detailed Description Text - DETX (91):

Mutant alpha-amylases were prepared having substitutions at one or more of positions V128E, H133Y, S187D and/or A209V generally according to the procedures provided in Examples 1-4 except that appropriate PCR primers were provided to effect the desired mutations. Amylases were purified to a point where wild type **Bacillus** licheniformis **.alpha.-amylase** showed a specific activity of 1087 LU/mg protein. Protein concentration was determined by absorption at 278 nm, using a Molar Extinction coefficient of wild type enzyme of 143,255 M.sup.-1 cm.sup.-1.

Other Reference Publication - OREF (2):

Declerck et al. (Jun. 1995) Hyperthermostable **mutants of Bacillus** licheniformis **alpha-amylase**: multiple amino acid replacement and molecular modeling. Protein Engineering 8(10): 1029-1037.

Other Reference Publication - OREF (56):

Mizuno et al., "Crystallization and Preliminary X-ray Studies of Wild Type and Catalytic-site **Mutant .alpha.-Amylase from bacillus** subtilis," J Mol Biol (1993) 234:1282-1283.

US-PAT-NO: 5928381

DOCUMENT-IDENTIFIER: US 5928381 A

TITLE: Use of an .alpha.-amylase modified to improve oxidation stability in a combined desizing and bleaching process

DATE-ISSUED: July 27, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Toft; Annette Hanne	Bagsv.ae butted.rd	N/A	N/A	DK
Marcher; Dorthe	Farum	N/A	N/A	DK
Pedersen; Hanne H.o	Lyngby	N/A	N/A	DK
slashed.st	Copenhagen .O slashed.	N/A	N/A	DK
Nilsson; Thomas Erik				

APPL-NO: 08/ 687399

DATE FILED: August 22, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a 35 U.S.C. 371 national application of PCT/DK94/00371 filed Oct. 5, 1994, which is incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0141/94	February 2, 1994

PCT-DATA:
APPL-NO: PCT/DK94/00371
DATE-FILED: October 5, 1994
PUB-NO: WO95/21247
PUB-DATE: Aug 10, 1995
371-DATE: Aug 22, 1996
102(E)-DATE:Aug 22, 1996

US-CL-CURRENT: 8/111, 435/263 , 510/305 , 510/392 , 510/393 , 8/101 , 8/107 , 8/138

ABSTRACT:

A process for simultaneously desizing and bleaching of a sized fabric containing starch or starch derivatives, which process comprises treating the fabric with a bleaching composition and an oxidation stable .alpha.-amylase.

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (11):
PCT/DK93/00230 discloses .alpha.-amylase **mutants having improved oxidation stability**. The mutants are indicated to be useful for desizing, but their use in a combined desizing and bleaching process is not mentioned.

Brief Summary Text - BSTX (25):
It is preferred that the oxidation stable **.alpha.-amylase** to be used in the present process is of microbial origin. More particularly, it is preferred that the **.alpha.-amylase** is derivable from a strain of **Bacillus**. Thus, **Bacillus .alpha.-amylases** exhibit in themselves a high heat **stability, and by being mutated as described above, the mutants may exhibit an even better stability,** especially in the presence of oxidizing agents.

Brief Summary Text - BSTX (46):
In the present context, a **mutant .alpha.-amylase** of particular interest is one, in which the methionine amino acid residue in position 197 in B. licheniformis **.alpha.-amylase** or the methionine amino acid residue in homologous positions in other **.alpha.-amylases** is exchanged. The concept of homologous positions or sequence homology of **.alpha.-amylases** has been explained e.g. in Nakajima, R. et al., 1986, Appl. Microbiol. Biotechnol. 23, 355-360 and Liisa Holm et al., 1990, Protein Engineering 3, 181-191. Sequence homology of **Bacillus .alpha.-amylases** from B. licheniformis, B. stearothermophilus and B. amyloliquefaciens are about 60%. This makes it possible to align the sequences in order to compare residues at homologous positions in the sequence. By such alignment of **.alpha.-amylase** sequences the number in each **.alpha.-amylase** sequence of the homologous residues can be found. The homologous positions will probably spatially be in the same position in a three dimensional structure (Greer, J., 1981, J. Mol. Biol. 153, 1027-1042), thus having analogous impact on specific functions of the enzyme in question. In relation to position 197 in B. licheniformis **.alpha.-amylase** the homologous positions in B. stearothermophilus **.alpha.-amylase** are positions 200 and 206, and the homologous position in B. amyloliquefaciens **.alpha.-amylase** is position 197. Experimentally it has been found that these **mutants** exhibit both an improved activity level and an improved stability in the presence of oxidizing agents.

US-PAT-NO: 5922083

DOCUMENT-IDENTIFIER: US 5922083 A
See image for Certificate of Correction

TITLE: Detergent composition comprising a mutant amylase enzyme and oxygen bleaching agent

DATE-ISSUED: July 13, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Biscarini; Lamberto	Bologna	N/A	N/A	IT
Trani; Marina	Rome	N/A	N/A	IT

APPL-NO: 08/ 930302

DATE FILED: October 3, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	95870031	April 3, 1995

PCT-DATA:
APPL-NO: PCT/US96/03340
DATE-FILED: March 12, 1996
PUB-NO: WO96/31583
PUB-DATE: Oct 10, 1996
371-DATE: Oct 3, 1997
102(E)-DATE:Oct 3, 1997

US-CL-CURRENT: 8/137, 510/283 , 510/284 , 510/305 , 510/311 , 510/320 , 510/357 , 510/372 , 510/374 , 510/375 , 510/396 , 510/490

ABSTRACT:

Soaking compositions are disclosed which comprise a bleach, a builder; an anionic surfactant, a proteolytic enzyme; and a stability enhanced amylase enzyme. A process of soaking fabrics is also disclosed, wherein said fabrics are immersed in a soaking liquor comprising water and an effective amount of the composition above.

13 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (16):
a stability enhanced amylase enzyme, said **stability enhancement being relative to the parent/non-mutant** form of said amylase enzyme.

Brief Summary Text - BSTX (63):
(a) An amylase according to the hereinbefore incorporated WO/94/02597, Novo Nordisk A/S, published Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine (preferably threonine), of the methionine residue located in position 197 of the **Bacillus** licheniformis **alpha-amylase,** known as TERMAMYL (R), or the homologous position variation of a similar parent amylase, such as **Bacillus** amyloliquefaciens, **Bacillus** subtilis, or **Bacillus** stearothermophilus;

Brief Summary Text - BSTX (64):
(b) Stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17, 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from **Bacillus** licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in Protease Enzymes" having U.S. Ser. No. 08/136,626, which are incorporated herein by reference.

US-PAT-NO: 5892013

DOCUMENT-IDENTIFIER: US 5892013 A

TITLE: Lipase variants

DATE-ISSUED: April 6, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Patkar; Shamkant Anant	Lyngby	N/A	N/A	DK
Gormsen; Erik	Virum	N/A	N/A	DK
Clausen; Ib Groth	Hiller.o slashed.d	N/A	N/A	DK
Okkels; Jens Sigurd	Frederiksberg	N/A	N/A	DK
Thellersen; Marianne	Frederiksberg	N/A	N/A	DK

APPL-NO: 08/ 488271

DATE FILED: September 5, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Ser. no. PCT/DK94/00162 filed Apr. 22, 1994, PCT/DK95/00079 filed Feb. 27, 1995 and of U.S. Ser. No. 08/434,904, filed May 1, 1995, now abandoned which is a continuation of Ser. No. 07/977,429 filed Feb. 22, 1993, now abandoned which is a continuation of PCT/DK91/00271 filed Sep. 13, 1991, which are incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	2194/90	September 13, 1990
DK	2195/90	September 13, 1990
DK	2196/90	September 13, 1990
DK	0466/93	April 23, 1993
DK	0217/94	February 22, 1994

US-CL-CURRENT: 536/23.2, 435/198 , 435/252.3 , 435/320.1 , 435/69.1 , 536/23.7

ABSTRACT:

The present invention relates to lipase variants which exhibit improved properties, detergent compositions comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants.

39 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (43):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), e.g., as described in WO 93/10249 the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (547):
Storage **stability of H. lanuginosa lipase variants** in liquid detergent

US-PAT-NO: 5869438

DOCUMENT-IDENTIFIER: US 5869438 A

TITLE: Lipase variants

DATE-ISSUED: February 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Patkar; Shamkant Anant	Lyngby	N/A	N/A	DK
Gormsen; Erik	Virum	N/A	N/A	DK
Okkels; Jens Sigurd	Frederiksberg	N/A	N/A	DK
Thellersen; Marianne	Frederiksberg	N/A	N/A	DK

APPL-NO: 08/ 479275

DATE FILED: June 7, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Ser. No. PCT/DK94/00162 filed Apr. 22, 1994, PCT/DK95/00079 filed Feb. 27, 1995 and of Ser. No. 08/434,904, filed May 1, 1995, now abandoned, which is a continuation of Ser. No. 07/977,429 filed Feb. 22, 1993, now abandoned, which is a continuation of PCT/DK91/00271 filed Sep. 13, 1991, which are incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	2194/90	September 13, 1990
DK	2195/90	September 13, 1990
DK	2196/90	September 13, 1990
DK	0466/93	April 23, 1993
DK	0217/94	February 22, 1994

US-CL-CURRENT: 510/226, 435/196 , 435/198 , 435/252.3 , 435/320.1 , 435/69.1 , 510/305 , 510/392 , 530/350 , 536/23.2 , 536/23.7

ABSTRACT:

The present invention relates to lipase variants which exhibit improved properties, detergent compositions comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants.

51 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Detailed Description Text - DETX (43):
In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding a **variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), e.g., as described in WO 93/10249 the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (515):
Storage **Stability of H. lanuginosa Lipase Variants** in Liquid Detergent

US-PAT-NO: 5858948

DOCUMENT-IDENTIFIER: US 5858948 A

TITLE: Liquid laundry detergent compositions comprising cotton soil release polymers and protease enzymes

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ghosh; Chanchal Kumar	Westchester	OH	N/A	N/A
Manohar; Sanjeev Krishnadas	Fairfield	OH	N/A	N/A
Gosselink; Eugene Paul	Cincinnati	OH	N/A	N/A
Watson; Randall Alan	Cincinnati	OH	N/A	N/A

APPL-NO: 08/ 841448

DATE FILED: April 22, 1997

US-CL-CURRENT: 510/300, 510/320 , 510/321 , 510/337 , 510/400 , 510/405 , 510/499 , 510/504 , 510/517 , 510/528 , 510/530

ABSTRACT:

Liquid laundry detergent compositions comprising water soluble and/or dispersible, modified polyamines having functionalized backbone moieties which provide cotton soil release benefits (optionally in combination with non-cotton soil release agents) and protease enzymes.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (61):

A prefered protease enzyme for use in the present invention is a bleach stable variant of Protease A (BPN'). This bleach stable variant of BPN' is a non-naturally occuring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. This bleach stable variant of BPN' is disclosed in EP 130,756 A, Jan. 9, 1985. Specifically Protease A-BSV is BPN' wherein the Gly at position 166 is replaced with Asn, Ser, Lys, Arg, His, Gln, Ala, or Glu; the Gly at position 169 is replaced with Ser; the Met at position 222 is replaced with Gln, Phe, Cys, His, Asn, Glu, Ala or Thr; or alternatively the Gly at position 166 is replaced with Lys, and the

Met at position 222 is replaced with Cys; or alternatively the Gly at position 169 is replaced with Ala and the Met at position 222 is replaced with Ala.

Brief Summary Text - BSTX (63):

A prefered protease enzyme for use in the present invention is Protease B. Protease B is a non-naturally occuring carbonyl hydrolase **variant having a different proteolytic activity, stability,** substrate specificity, pH profile and/or performance characteristic as compared to the precursor carbonyl hydrolase from which the amino acid sequence of the variant is derived. Protease B is a variant of BPN' in which tyrosine is replaced with leucine at position +217 and as further disclosed in EP 303,761 A, Apr. 28, 1987 and EP 130,756 A, Jan. 9, 1985.

Brief Summary Text - BSTX (252):

Amylases suitable herein include, for example, **.alpha.-amylases** described in GB 1,296,839 to Novo; RAPIDASE.RTM., International Bio-Synthetics, Inc. and TERMAMYL.RTM., Novo. FUNGAMYL.RTM. from Novo is especially useful. Engineering of enzymes for improved stability, e.g., oxidative stability, is known. See, for example J. Biological Chem., Vol. 260, No. 11, Jun. 1985, pp 6518-6521. Certain preferred embodiments of the present compositions can make use of amylases having improved stability in detergents, especially improved oxidative stability as measured against a reference-point of TERMAMYL.RTM. in commercial use in 1993. These preferred amylases herein share the characteristic of being "stability-enhanced" amylases, characterized, at a minimum, by a measurable improvement in one or more of: oxidative stability, e.g., to hydrogen peroxide/tetraacetylethylenediamine in buffered solution at pH 9-10; thermal stability, e.g., at common wash temperatures such as about 600.degree. C.; or alkaline stability, e.g., at a pH from about 8 to about 11, measured versus the above-identified reference-point amylase. Stability can be measured using any of the art-disclosed technical tests. See, for example, references disclosed in WO 9402597. Stability-enhanced amylases can be obtained from Novo or from Genencor International. One class of highly preferred amylases herein have the commonality of being derived using site-directed mutagenesis from one or more of the Baccillus amylases, especially the **Bacillus .alpha.-amylases,** regardless of whether one, two or multiple amylase strains are the immediate precursors. Oxidative stability-enhanced amylases vs. the above-identified reference amylase are preferred for use, especially in bleaching, more preferably oxygen bleaching, as distinct from chlorine bleaching, detergent compositions herein. Such preferred amylases include (a) an amylase according to the hereinbefore incorporated WO 9402597, Novo, Feb. 3, 1994, as further illustrated by a **mutant** in which substitution is made, using alanine or threonine, preferably threonine, of the methionine residue located in position 197 of the B. licheniformis **alpha-amylase,** known as TERMAMYL.RTM., or the homologous position variation of a similar parent amylase, such as B. amyloliquefaciens, B. subtilis, or B. stemirothermophilus; (b) stability-enhanced amylases as described by Genencor International in a paper entitled "Oxidatively Resistant **alpha-Amylases**" presented at the 207th American Chemical Society National Meeting, Mar. 13-17 1994, by C. Mitchinson. Therein it was noted that bleaches in automatic dishwashing detergents inactivate **alpha-amylases** but that improved oxidative stability amylases have been made by Genencor from B.

licheniformis NCIB8061. Methionine (Met) was identified as the most likely residue to be modified. Met was substituted, one at a time, in positions 8, 15, 197, 256, 304, 366 and 438 leading to specific **mutants,** particularly important being M197L and M197T with the M197T **variant** being the most stable expressed **variant**. Stability was measured in CASCADE.RTM. and SUNLIGHT.RTM.; (c) particularly preferred amylases herein include amylase **variants** having additional modification in the immediate parent as described in WO 9510603 A and are available from the assignee, Novo, as DURAMYL.RTM.. Other particularly preferred oxidative stability enhanced amylase include those described in WO 9418314 to Genencor International and WO 9402597 to Novo. Any other oxidative stability-enhanced amylase can be used, for example as derived by site-directed mutagenesis from known chimeric, hybrid or simple **mutant** parent forms of available amylases. Other preferred enzyme modifications are accessible. See WO 9509909 A to Novo.

US-PAT-NO: 5849549

DOCUMENT-IDENTIFIER: US 5849549 A

TITLE: Oxidatively stable alpha-amylase

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Franciso	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A

APPL-NO: 08/ 468698

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATION

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending, which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993, abandoned.

US-CL-CURRENT: 435/99, 435/202 , 536/23.2

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

2 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

Brief Summary Text - BSTX (2):

The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The **mutant enzymes of the present invention exhibit altered stability**/activity profiles including but not limited to altered oxidative stability, altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (13):

The alpha-amylase **mutants of the present invention, in general, exhibit altered oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. **Mutant enzymes having enhanced oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced oxidative stability may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby **mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability** at high pH cleaning product conditions. The **mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability** at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase.

Brief Summary Text - BSTX (15):

The preferred **alpha-amylase mutants** of the present invention are derived from a **Bacillus** strain such as B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus, and most preferably from **Bacillus** licheniformis.

Drawing Description Text - DRTX (14):
FIG. 10 shows thermal **stability f M197X variants** at pH 5.0, 5 mM CaCl.sub.2 at 95.degree. C. for 5 mins.

Drawing Description Text - DRTX (20):
FIG. 15 shows heat **stability of M15X variants** at 90.degree. C., pH 5.0, 5 mM CaCl.sub.2, 5 mins.

Detailed Description Text - DETX (10):
Host strains (or cells) useful in the present invention generally are procaryotic or eucaryotic hosts and include any transformable microorganism in which the expression of **alpha-amylase** can be achieved. Specifically, host strains of the same species or genus from which the **alpha-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably an **alpha-amylase** negative **Bacillus** strain (genes deleted) and/or an **alpha-amylase** and protease deleted **Bacillus** strain such as **Bacillus** subtilis strain BG2473 (.DELTA.amyE,.DELTA.apr,.DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **alpha-amylase and its variants (mutants)** or expressing the desired **alpha-amylase**.

Detailed Description Text - DETX (72):
All M15X variants were propagated in Bacillus subtilis and the expression level monitored as shown in FIG. 13. The amylase was isolated and partially purified by a 20-70% ammonium sulfate cut. The specific activity of these variants on the soluble substrate was determined as per Example 3 (FIG. 14). Many of the M15X amylases have specific activities greater than that of Spezyme.RTM. AA20. A benchtop heat **stability assay was performed on the variants** by heating the amylase at 90.degree. C. for 5 min. in 50 mM acetate buffer pH 5 in the presence of 5 mM CaCl.sub.2 (FIG. 15). Most of the variants performed as well as Spezyme.RTM. AA20 in this assay. Those **variants that exhibited reasonable stability** in this assay (reasonable stability defined as those that retained at least about 60% of Spezyme.RTM. AA20's heat stability) were tested for specific activity on starch and for liquefaction performance at pH 5.5. The most interesting of those mutants are shown in FIG. 16. M15D, N and T, along with L, outperformed Spezyme.RTM. AA20 in liquefaction at pH 5.5 and have increased specific activities in both the soluble substrate and starch hydrolysis assays.

Claims Text - CLTX (2):
adding to the slurry an effective amount of a **mutant alpha-amylase** derived from **Bacillus, the alpha-amylase mutant** comprising a substitution of threonine, leucine, asparagine or aspartic acid for a methionine residue corresponding to M15 in **Bacillus** licheniformis **alpha-amylas** ; optionally adding an effective amount of an antioxidant to the slurry; and reacting the slurry for an

appropriate time and at an appropriate temperature to liquefy the starch.

Claims Text - CLTX (4):
adding to the slurry an effective amount of a **mutant alpha-amylase** derived from **Bacillus, the alpha-amylase mutant** comprising a substitution of leucine or alanine for a methionine residue corresponding to M197 in **Bacillus** licheniformis **alpha-amylase**; optionally adding an effective amount of an antioxidant to the slurry; and reacting the slurry for an appropriate time and at an appropriate temperature to liquefy the starch.

US-PAT-NO: 5830837

DOCUMENT-IDENTIFIER: US 5830837 A
See image for Certificate of Correction

TITLE: Amylase variants

DATE-ISSUED: November 3, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben Vedel	K.o slashed.benhavn	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Thellersen; Marianne	Frederiksberg	N/A	N/A	DK
Van der Zee; Pia	Virum	N/A	N/A	DK

APPL-NO: 08/ 343804

DATE FILED: November 22, 1994

US-CL-CURRENT: 510/226, 435/202 , 435/203 , 435/204 , 435/252.3 , 435/320.1 , 435/69.1 , 510/392 , 536/23.1 , 536/23.7

ABSTRACT:

A variant of a parent .alpha.-amylase enzyme having an improved washing and/or dishwashing performance as compared to the parent enzyme, wherein one or more amino acid residues of the parent enzyme have been replaced by a different amino acid residue and/or wherein one or more amino acid residues of the parent .alpha.-amylase have been deleted and/or wherein one or more amino acid residues have been added to the parent .alpha.-amylase enzyme, provided that the variant is different from one in which the methionine residue in position 197 of a parent B. licheniformis .alpha.-amylase has been replaced by alanine or threonine, as the only modification being made. The variant may be used for washing and dishwashing.

42 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX (9):

WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase

mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Brief Summary Text - BSTX (13):

EP 525 610 relates to **mutant enzymes having an improved stability** towards ionic tensides. The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations have not been specified.

Brief Summary Text - BSTX (14):

WO 94/02597 which was unpublished at the priority dates of the present invention, discloses novel .alpha.-amylase **mutants which exhibit an improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Brief Summary Text - BSTX (67):

Thus, in accordance with the present invention it has surprisingly been found possible to use the high degree of amino acid sequence homology observed between the **.alpha.-amylases** produced by the **Bacillus** spp. B. licheniformis, B. amyloliquefaciens and B. stearothermophilus to prepare **.alpha.-amylase variants** having improved washing and/or dishwashing performance. More specifically, the **variants** are prepared on the basis of modification of one or more specific amino acid residues to one or more amino acid residues present in a corresponding or homologous position of the other homologous **.alpha.-amylases**.

Brief Summary Text - BSTX (85):

In particular, the region defined by amino acid residues 29.gtoreq.35 of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 2 comprises a large number of positions in which no homology exists between the various **Bacillus .alpha.-amylases**. Accordingly, the B. licheniformis **.alpha.-amylase variant** of the invention may be a **variant** in which at least one amino acid residue located in position 29.gtoreq.35 of the parent **.alpha.-amylase** has been substituted or deleted, or in which at least one amino acid has been added to the parent **.alpha.-amylase** within the amino acid segment located in position 29-35.

Brief Summary Text - BSTX (172):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (200):

The storage **stability of .alpha.-amylase variant** amyL variant III+M197T was determined by adding the variant and its parent .alpha.-amylase, respectively, to the detergent in an amount corresponding to a dosage of 0.5 mg enzyme protein per liter of washing liquor (3 liters in the main wash) together with 12 g of detergent in each wash (1.5 mg enzyme protein). The mixtures were stored at 30.degree. C./60% relative humidity (r.h.) for 0, 1, 2, 3, 4, and 6 weeks. After storage the analytical activity of the samples were determined as well as the performance. The performance was tested by using the whole content of each storage glass (containing enzyme and detergent) in each wash. The soil was corn starch on plates and glasses, and the dishwashing was carried out at 55.degree. C., using a Cylinda 770 machine. The storage stability is illustrated in FIGS. 10 and 11. amyL variant III+M197T was significantly more stable than its parent enzyme.

US-PAT-NO: 5824532

DOCUMENT-IDENTIFIER: US 5824532 A

TITLE: Oxidativley stable alpha-amylase

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A

APPL-NO: 08/ 468220

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATIONS

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993 now abandoned.

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/204 , 435/252.3 , 435/252.31 , 435/320.1 , 435/71.2 , 536/23.2 , 536/23.7

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

11 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):
Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such **mutant alpha-amylases have altered oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

Brief Summary Text - BSTX (2):
The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The **mutant enzymes of the present invention exhibit altered stability**/activity profiles including but not limited to altered oxidative stability, altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (13):
The alpha-amylase **mutants of the present invention, in general, exhibit altered oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. **Mutant enzymes having enhanced oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced oxidative stability may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby **mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability** at high pH cleaning product conditions. The **mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability** at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase.

Brief Summary Text - BSTX (15):
The preferred **alpha-amylase mutants** of the present invention are derived from a **Bacillus** strain such as B. licheniformis, B. amyloliquefaciens, and B. stearothennophilus, and most preferably from **Bacillus** licheniformis.

Drawing Description Text - DRTX (14):
FIG. 10 shows thermal **stability f M197X variants** at pH 5.0, 5mM CaCl.sub.2 at 95.degree. C. for 5 mins.

Drawing Description Text - DRTX (20):
FIG. 15 shows heat **stability of M15X variants** at 90.degree. C., pH 5.0, 5 mM CaCl.sub.2, 5 mins.

Detailed Description Text - DETX (10):
Host strains (or cells) useful in the present invention generally are procaryotic or eucaryotic hosts and include any transformable microorganism in which the expression of **alpha-amylase** can be achieved. Specifically, host strains of the same species or genus from which the **alpha-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably an **alpha-amylase** negative **Bacillus** strain (genes deleted) and/or an **alpha-amylase** and protease deleted **Bacillus** strain such as **Bacillus** subtilis strain BG2473 (.DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **alpha-amylase and its variants (mutants)** or expressing the desired **alpha-amylase**.

Detailed Description Text - DETX (71):
All M15X variants were propagated in Bacillus subtilis and the expression level monitored as shown in FIG. 13. The amylase was isolated and partially purified by a 20-70% ammonium sulfate cut. The specific activity of these variants on the soluble substrate was determined as per Example 3 (FIG. 14). Many of the M15X amylases have specific activities greater than that of Spezyme.RTM. AA20. A benchtop heat **stability assay was performed on the variants** by heating the amylase at 90.degree. C. for 5 min. in 50 mM acetate buffer pH 5 in the presence of 5 mM CaCl.sub.2 (FIG. 15). Most of the variants performed as well as Spezyme.RTM. AA20 in this assay. Those **variants that exhibited reasonable stability** in this assay (reasonable stability defined as those that retained at least about 60% of Spezyme.RTM. AA20's heat stability) were tested for specific activity on starch and for liquefaction performance at pH 5.5. The most interesting of those mutants are shown in FIG. 16. M15D, N and T, along with L, out performed Spezyme.RTM. AA20 in liquefaction at pH 5.5 and have increased specific activities in both the soluble substrate and starch hydrolysis assays.

Claims Text - CLTX (1):
1. DNA encoding a **mutant alpha-amylase, the mutant alpha-amylase** derived from **Bacillus** and comprising:

US-PAT-NO: 5801043

DOCUMENT-IDENTIFIER: US 5801043 A
See image for Certificate of Correction

TITLE: Amylase variants

DATE-ISSUED: September 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisg.ang.rd-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben Vedel	K.o slashed.benhavn	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Thellersen; Marianne	Frederiksberg	N/A	N/A	DK
Van der Zee; Pia	Virum	N/A	N/A	DK

APPL-NO: 08/ 459610

DATE FILED: June 2, 1995

PARENT-CASE:

This application is a Continuation application of co-pending application Ser. No. 08/343,804, filed as PCT/DK94/00370, Oct. 5, 1994.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1133	October 8, 1993
DK	0140	February 2, 1994

US-CL-CURRENT: 435/252.3, 435/202 , 435/203 , 435/204 , 435/252.31 , 435/320.1 , 435/69.1 , 536/23.2 , 536/23.7

ABSTRACT:

A variant of a parent .alpha.-amylase enzyme having an improved washing and/or dishwashing performance as compared to the parent enzyme, wherein one or more amino acid residues of the parent enzyme have been replaced by a different amino acid residue and/or wherein one or more amino acid residues of the parent .alpha.-amylase have been deleted and/or wherein one or more amino acid residues have been added to the parent .alpha.-amylase enzyme, provided that the variant is different from one in which the methionine residue in position 197 of a parent B. licheniformis .alpha.-amylase has been replaced by alanine or threonine, as the only modification being made. The variant may be used for washing and dishwashing.

36 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX (9):
WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations: H133Y or H133Y+T149I. Another suggested mutation is A111T.

Brief Summary Text - BSTX (13):
EP 525 610 relates to **mutant enzymes having an improved stability** towards ionic tensides. The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations have not been specified.

Brief Summary Text - BSTX (14):
WO 94/02597 which was unpublished at the priority dates of the present invention, discloses novel .alpha.-amylase **mutants which exhibit an improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Brief Summary Text - BSTX (62):
Thus, in accordance with the present invention it has surprisingly been found possible to use the high degree of amino acid sequence homology observed between the **.alpha.-amylases** produced by the **Bacillus** spp. B. licheniformis, B. amyloliquefaciens and B. stearothermophilus to prepare **.alpha.-amylase variants** having improved washing and/or dishwashing performance. More specifically, the **variants** are prepared on the basis of modification of one or more specific amino acid residues to one or more amino acid residues present in a corresponding or homologous position of the other homologous **.alpha.-amylases**.

Brief Summary Text - BSTX (80):

In particular, the region defined by amino acid residues 29-35 of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 2 comprises a large number of positions in which no homology exists between the various **Bacillus .alpha.-amylases**. Accordingly, the B. licheniformis **.alpha.-amylase variant** of the invention may be a **variant** in which at least one amino acid residue located in position 29-35 of the parent **.alpha.-amylase** has been substituted or deleted, or in which at least one amino acid has been added to the parent **.alpha.-amylase** within the amino acid segment located in position 29-35.

Brief Summary Text - BSTX (167):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xylA and xylB genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (151):

The storage **stability of .alpha.-amylase variant** amyL variant III+M197T was determined by adding the variant and its parent .alpha.-amylase, respectively, to the detergent in an amount corresponding to a dosage of 0.5 mg enzyme protein per liter of washing liquor (3 liters in the main wash) together with 12 g of detergent in each wash (1.5 mg enzyme protein). The mixtures were stored at 30.degree. C./60% relative humidity (r.h.) for 0, 1, 2, 3, 4, and 6 weeks. After storage the analytical activity of the samples were determined as well as the performance. The performance was tested by using the whole content of each storage glass (containing enzyme and detergent) in each wash. The soil was corn starch on plates and glasses, and the dishwashing was carried out at. 55.degree. C., using a Cylinda 770 machine. The storage **stability is illustrated in FIGS. 10 and 11 amyL variant** III+M197T was significantly more stable than its parent enzyme.

US-PAT-NO: 5763385

DOCUMENT-IDENTIFIER: US 5763385 A

TITLE: Modified .alpha.-amylases having altered calcium binding properties

DATE-ISSUED: June 9, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bott; Richard R.	Burlingame	CA	N/A	N/A
Shaw; Andrew	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 645971

DATE FILED: May 14, 1996

US-CL-CURRENT: 510/392, 435/201 , 435/202 , 435/836

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which a new calcium binding site is modified by chemically or genetically altering residues associated with that calcium binding site. The novel .alpha.-amylases have altered performance characteristics, such as low pH starch hydrolysis performance, stability and activity profiles.

28 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX (7):

In PCT Publication No. WO95/10603, **.alpha.-amylase variants** are disclosed which have improved laundry or dishwashing performance and comprise a mutation other than a single mutation at position M197 in **Bacillus** licheniformis **.alpha.-amylase**.

Brief Summary Text - BSTX (8):

In PCT Publication No. WO94/02597, a **mutant .alpha.-amylase having improved xidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (9):

In PCT Publication No. WO94/18314, a **mutant .alpha.-amylase having impr ved xidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (27):

The modified .alpha.-amylases according to the present invention will provide several important advantages when compared to prior art .alpha.-amylases. For example, one advantage is found in variants having increased activity at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is found in **variants having increased high pH and oxidative stability** which facilitates their use in detergents. Yet another advantage is provided by **variants having improved stability** in the absence or low concentration of calcium ion. The objects and attendant advantages of the present invention will be made more clear in the following detailed description and examples.

Detailed Description Text - DETX (5):

"Host strain" or "host cell" means a suitable host for, e.g., an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (e.g., .DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (12):

A wide variety of host cells are also useful in expressing the DNA sequences of this invention and are contemplated herein. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of E. coli, Pseudomonas, **Bacillus,** Streptomyces, various fungi, e.g., Trichoderma or Aspergillus, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (15):

The discovery of the CalB binding site in a **Bacillus .alpha.-amylase** by

Applicants has enabled Applicants to develop **mutant .alpha.-amylases** having altered performance, and particularly altered stability. For example, general principles for stabilization of protein structure may be applied to the region around the CalB site. Additionally, strategies specifically designed to improve calcium binding at the CalB site may be implemented to increase the stability of the enzyme. Preferably, such modifications are within 15 angstroms of the center of mass of the calcium bound to the CalB binding site, more preferably within 10 angstroms of the center of mass of the calcium bound to the CalB binding site.

Detailed Description Text - DETX (18):

The segments of the .alpha.-amylase polypeptide chain which comprise the CalB binding site include residues 290-309, 339-347, 402-411, 426-436 and 472-477. These polypeptide segments comprise the CalB binding site. Accordingly, regiospecific random mutations in these regions would be expected to yield **variants that modulate the stability** of .alpha.-amylase via modulation of the affinity of calcium at this site.

Detailed Description Text - DETX (33):

Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Pat. No. Re 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Pat. No. Re 34,606.

Other Reference Publication - OREF (2):

Declerck, et al., "Hyperthermostable **mutants of Bacillus** licheniformis **.alpha.-amylase**: multiple amino acid replacements and molecular modelling," Protein Engineering, (1995) 8(10):1029-1037.

US-PAT-NO: 5756714

DOCUMENT-IDENTIFIER: US 5756714 A

TITLE: Method for liquefying starch

DATE-ISSUED: May 26, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Antrim; Richard L.	Solon	IA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 411038

DATE FILED: March 27, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/401,325 filed Mar. 9, 1995, now abandoned and which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 536/102, 435/202 , 435/203 , 435/204 , 435/205 , 435/96 , 435/99

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of treating the starch prior to or simultaneously with liquefying the starch to inactivate and/or remove the enzyme inhibiting composition present in the starch and form treated starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Effective means to treat the starch include the addition of a phytate degrading enzyme and heat treatment, optionally followed by filtration or centrifugation, of granular starch or a starch solution.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (17):

In PCT publication No. 94/18314, a **mutant-amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (36):

"**.alpha.-amylase**" means an enzymatic activity which cleaves or hydrolyzes the .alpha.(1-4) glycosidic bond, e.g., that in starch, amylopectin or amylose polymers. Suitable **.alpha.-amylases** are the naturally occurring **.alpha.-amylases** as well as recombinant or **mutant** amylases which are useful in liquefaction of starch. Preferred amylases in the present invention are **.alpha.-amylases** derived from **Bacillus, and particularly Bacillus** licheniformis, **Bacillus** amyloliquefaciens or **Bacillus** stearothermophilus.

US-PAT-NO: 5753460

DOCUMENT-IDENTIFIER: US 5753460 A

TITLE: Amylase variants

DATE-ISSUED: May 19, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bisgard-Frantzen; Henrik	Lyngby	N/A	N/A	DK
Borchert; Torben Vedel	K.o slashed.benhavn N	N/A	N/A	DK
Svendsen; Allan	Birker.o slashed.d	N/A	N/A	DK
Thellersen; Marianne	Frederiksberg C	N/A	N/A	DK
Van der Zee; Pia	Virum	N/A	N/A	DK

APPL-NO: 08/ 720899

DATE FILED: October 10, 1996

PARENT-CASE:

This is a divisional application of co-pending application Ser. No. 08/459,610, filed Jun. 2, 1995 which is a continuation of co-pending application Ser. No. 08/343,804, filed Nov. 22, 1994, which is a continuation of application Ser. No. 08/321,271 filed Oct. 11, 1994, now abandoned.

US-CL-CURRENT: 435/69.1, 435/202 , 435/203 , 435/204 , 435/252.3 , 435/320.1 , 510/226 , 536/23.2 , 536/23.7

ABSTRACT:

A variant of a parent .alpha.-amylase enzyme having an improved washing and/or dishwashing performance as compared to the parent enzyme, wherein one or more amino acid residues of the parent enzyme have been replaced by a different amino acid residue and/or wherein one or more amino acid residues of the parent .alpha.-amylase have been deleted and/or wherein one or more amino acid residues have been added to the parent .alpha.-amylase enzyme, provided that the variant is different from one in which the methionine residue in position 197 of a parent B. licheniformis .alpha.-amylase has been replaced by alanine or threonine, as the only modification being made. The variant may be used for washing and dishwashing.

31 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

---------- KWIC ---------

Brief Summary Text - BSTX (9):
WO 91/00353 discloses .alpha.-amylase mutants which differ from their parent .alpha.-amylase in at least one amino acid residue. The .alpha.-amylase mutants disclosed in said patent application are stated to exhibit improved properties for application in the degradation of starch and/or textile desizing due to their amino acid substitutions. Some of the **mutants exhibit improved stability,** but no improvements in enzymatic activity were reported or indicated. The only mutants exemplified are prepared from a parent B. licheniformis .alpha.-amylase and carry one of the following mutations suggested mutation is A111T.

Brief Summary Text - BSTX (13):
EP 525 610 relates to **mutant enzymes having an improved stability** towards ionic tensides. The mutant enzymes have been produced by replacing an amino acid residue in the surface part of the parent enzyme with another amino acid residue. The only mutant enzyme specifically described in EP 525 610 is a protease. Amylase is mentioned as an example of an enzyme which may obtain an improved stability towards ionic tensides, but the type of amylase, its origin or specific mutations have not been specified.

Brief Summary Text - BSTX (14):
WO 94/02597 which was unpublished at the priority dates of the present invention, discloses novel .alpha.-amylase **mutants which exhibit an improved stability** and activity in the presence of oxidizing agents. In the mutant .alpha.-amylases, one or more methionine residues have been replaced with amino acid residues different from Cys and Met. The .alpha.-amylase mutants are stated to be useful as detergent and/or dishwashing additives as well as for textile desizing.

Brief Summary Text - BSTX (67):
Thus, in accordance with the present invention it has surprisingly been found possible to use the high degree of amino acid sequence homology observed between the **.alpha.-amylases** produced by the **Bacillus** spp. B. licheniformis, B. amyloliquefaciens and B. stearothermophilus to prepare **.alpha.-amylase variants** having improved washing and/or dishwashing performance. More specifically, the **variants** are prepared on the basis of modification of one or more specific amino acid residues to one or more amino acid residues present in a corresponding or homologous position of the other homologous **.alpha.-amylases**.

Brief Summary Text - BSTX (85):
In particular, the region defined by amino acid residues 29-35 of the B. licheniformis **.alpha.-amylase** comprising the amino acid sequence shown in SEQ ID No. 2 comprises a large number of positions in which no homology exists between the various **Bacillus .alpha.-amylases**. Accordingly, the B.

licheniformis **.alpha.-amylase variant** of the invention may be a **variant** in which at least one amino acid residue located in position 29-35 of the parent **.alpha.-amylase** has been substituted or deleted, or in which at least one amino acid has been added to the parent **.alpha.-amylase** within the amino acid segment located in position 29-35.

Brief Summary Text - BSTX (174):

In the vector, the DNA sequence should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the DNA sequence encoding an **.alpha.-amylase variant** of the invention, especially in a bacterial host, are the promoter of the lac operon of E. coli, the Streptomyces coelicolor agarase gene dagA promoters, the promoters of the **Bacillus** licheniformis **.alpha.-amylase** gene (amyL), the promoters of the **Bacillus** stearothermophilus maltogenic amylase gene (amyM), the promoters of the **Bacillus** Amyloliquefaciens **.alpha.-amylase** (amyQ), the promoters of the **Bacillus** subtilis xy1A and xy1B genes etc. For transcription in a fungal host, examples of useful promoters are those derived from the gene encoding A. oryzae TAKA amylase, Rhizomucor miehei aspartic proteinase, A. niger neutral **.alpha.-amylase,** A. niger acid stable **.alpha.-amylase,** A. niger glucoamylase, Rhizomucor miehei lipase, A. oryzae alkaline protease, A. oryzae triose phosphate isomerase or A. nidulans acetamidase.

Detailed Description Text - DETX (161):

The storage **stability of .alpha.-amylase variant** amyL variant III+M197T was determined by adding the variant and its parent .alpha.-amylase, respectively, to the detergent in an amount corresponding to a dosage of 0.5 mg enzyme protein per liter of washing liquor (3 liters in the main wash) together with 12 g of detergent in each wash (1.5 mg enzyme protein). The mixtures were stored at 30.degree. C./60% relative humidity (r.h.) for 0, 1, 2, 3, 4, and 6 weeks. After storage the analytical activity of the samples were determined as well as the performance. The performance was tested by using the whole content of each storage glass (containing enzyme and detergent) in each wash. The soil was corn starch on plates and glasses, and the dishwashing was carried out at 55.degree. C., using a Cylinda 770 machine. The storage stability is illustrated in FIGS. 10 and 11. amyL variant III+M197T was significantly more stable than its parent enzyme.

US-PAT-NO: 5741767

DOCUMENT-IDENTIFIER: US 5741767 A

TITLE: Peracid based dishwashing detergent composition

DATE-ISSUED: April 21, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nicholson; John Richard	Ramsey	NJ	N/A	N/A
Secemski; Isaac Israel	Teaneck	NJ	N/A	N/A
Rick; Deborah Sue	New Milford	NJ	N/A	N/A
Raible; Duane Anthony	Park Ridge	NJ	N/A	N/A

APPL-NO: 08/ 558994

DATE FILED: November 16, 1995

US-CL-CURRENT: 510/220, 134/25.2 , 510/221 , 510/223 , 510/226 , 510/227 , 510/229 , 510/230 , 510/372 , 510/374 , 510/375 , 510/392 , 510/393 , 510/441

ABSTRACT:

A warewashing composition for a machine dishwasher and a method of using it is described. The composition comprises effective amounts of an organic peroxy acid, and an amylase enzyme which, when incubated at 55.degree. C. in a solution of 2 mM sodium citrate, 1 mM epsilon phthalimidoperoxyhexanoic acid in 36 ppm water at pH 8.0, has a half-life of two minutes or greater based on an activity vs. time plot obtained via monitoring color development at 405 nm of solution samples incubated with p-nitrophenyl-.alpha.-D-maltoheptaoside as substrate and gluco amylase and .alpha.-glucosidase as coupled enzymes; and 1% to 75% by weight of a builder. A 1% aqueous solution of the composition must have a pH of less than 10.

29 Claims, 0 Drawing figures

Exemplary Claim Number: 1,16

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Brief Summary Text - BSTX (41):

A preferred embodiment of the **.alpha.-amylase** is characterized by the fact that the **alpha amylase** is a Baciflus **.alpha.-amylase**. Preferred embodiments of the bleach resistant **.alpha.-amylas** are characterized by the fact that the **.alpha.-amylase is Bacillus** licheniformis **.alpha.-amylase,** B. amyloliquefaciens **.alpha.-amylase** and B. stearothermophilus **.alpha.-amylase,** and furthermore

Aspergillus niger **.alpha.-amylase**. It has been found that this entire group of **mutant .alpha.-amylases** exhibit a half-life of greater than two minutes under the test conditions outlined in the "Summary of the Invention".

Brief Summary Text - BSTX (43):

A preferred embodiment of the **mutant .alpha.-amylase** is characterized by the fact that the methionine amino acid residue in position 197 in B. licheniformis **.alpha.-amylase** or the methionine amino acid residue in homologous positions in other **.alpha.-amylases** is exchanged. The concept of homologous positions or sequence homology of **.alpha.-amylase** has been explained e.g. in Nakajima, R. et al., 1986, Appl. Microbiol. Biotechnol. 23, 355-360 and Liisa Holm et al., 1990, Protein Engineering 3, 181-191. Sequence homology of **Bacillus .alpha.-amylases** from B. licheniforms, B. stearothermophilus and B. amyloliquefaciens are about 60%. This makes it possible to align the sequences in order to compare residues at homologous positions in the sequence. By such alignment of **.alpha.-amylase** sequences the number in each **.alpha.-amylase** sequence of the homologous residues can be found. The homologous positions will probably spatially be in the same positions in a three dimensional structure (Greer, J., 1981, J. Mol. Biol. 153, 1027-1042) thus having analogous impact on specific functions of the enzyme in question. In relation to position 197 in B. licheniformis **.alpha.-amylase,** the homologous positions in B. stearothermophils **.alpha.-amylase** are positions 200 and 206, and the homologous position in B. amyloliquefaciens **.alpha.-amylase** is position 197. Experimentally it has been found that these mutuants exhibit both an improved activity level and an improved stability in the presence of oxidizing agents.

Detailed Description Text - DETX (18):

Chlorine bleach has a devastating impact on the **stability of both amylase variants** and so the cleaning results are poor in both cases. There is a big improvement in enzyme stability when the bleach is hydrogen peroxide. However, the starch removal performance of both enzymes remained essentially unchanged relative to the composition with no bleach described in Example 2. Thus, in the presence of hydrogen peroxide at pH 10, it is the conventional amylase, not the bleach-resistant amylase that gives the better starch removal performance.

US-PAT-NO: 5736499

DOCUMENT-IDENTIFIER: US 5736499 A

TITLE: Mutant A-amylase

DATE-ISSUED: April 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Requadt; Carol Ann	Tiburon	CA	N/A	N/A
Ropp; Traci Helen	San Francisco	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 468700

DATE FILED: June 6, 1995

US-CL-CURRENT: 510/392, 435/201 , 435/202 , 435/203 , 435/204 , 510/393 , 510/530

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more asparagine residues are substituted with a different amino acid or deleted. The disclosed .alpha.-amylase enzymes show altered or improved low pH starch hydrolysis performance, stability and activity profiles.

25 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (17):

In PCT publication No. WO 94/18314, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more of the methionine, tryptophan, cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Drawing Description Text - DRTX (10):
FIG. 9 illustrates a schematic of the PCR method used to produce the **mutant** oligonucleotide primers corresponding to **.alpha.-amylase** derived from **Bacillus** lichenformis.

Drawing Description Text - DRTX (11):
FIG. 10 illustrates a graph derived from a statistical analysis of **variant** enzyme according to the invention, M 15T/N188S, compared to wild type **Bacillus** lichenformis **.alpha.-amylase** in starch liquefaction at 107.degree. C., 60 ppm calcium and varying pH.

Drawing Description Text - DRTX (12):
FIG. 11 illustrates a graph derived from a statistical analysis of the performance of a **variant** enzyme according to the invention, M15T/N188S, compared to wild type **Bacillus** lichenformis **.alpha.-amylase** in starch liquefaction at 107.degree. C., pH 6.0 and varying calcium concentration.

Drawing Description Text - DRTX (13):
FIG. 12 illustrates a graph derived from a statistical analysis of the performance of a **variant** enzyme according to the invention, M15T/N188S, compared to wild type **Bacillus** lichenformis **.alpha.-amylase** in starch liquefaction at pH 6.0, 60 ppm calcium and varying temperature.

Detailed Description Text - DETX (6):
"Host strain" or "host cell" means a suitable host for an expression vector comprising DNA encoding the **.alpha.-amylase** according to the present invention. Host cells useful in the present invention are generally procaryotic or eucaryotic hosts, including any transformable microorganism in which the expression of **.alpha.-amylase** according to the present invention can be achieved. Specifically, host strains of the same species or genus from which the **.alpha.-amylase** is derived are suitable, such as a **Bacillus** strain. Preferably, an **.alpha.-amylase** negative **Bacillus** strain (genes deleted) and/or an **.alpha.-amylase** and protease deleted **Bacillus** strain (.DELTA.amyE, .DELTA.apr, .DELTA.npr) is used. Host cells are transformed or transfected with vectors constructed using recombinant DNA techniques. Such transformed host cells are capable of either replicating vectors encoding the **.alpha.-amylase and its variants (mutants)** or expressing the desired **.alpha.-amylase**.

Detailed Description Text - DETX (21):
Embodiments of the present invention which comprise a combination of the **.alpha.-amylase** according to the present invention with protease enzymes preferably include oxidatively stable proteases such as those described in U.S. Pat. No. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM

(Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease **mutants** (oxidatively stable proteases), and particularly such **mutants** having a substitution for the methionine at a position equivalent to M222 in **Bacillus** amyloliquefaciens, are described in U.S. Pat. No. Re. 34,606.

Detailed Description Text - DETX (23):

A wide variety of host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of E. coil, Pseudomonas, **Bacillus,** Streptomyces, various fungi, yeast and animal cells. Preferably, the host expresses the **.alpha.-amylase** of the present invention extracellularly to facilitate purification and downstream processing. Expression and purification of the **mutant .alpha.-amylase** of the invention may be effected through art-recognized means for carrying out such processes.

Detailed Description Text - DETX (24):

The improved **.alpha.-amylases** according to the present invention provide several important advantages when compared to wild type **Bacillus .alpha.-amylases**. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and oxidative stability which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of **.alpha.-amylase** according to the invention provide superior performance when compared to wild type **Bacillus** lichenformis **.alpha.-amylase** due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this **variant**. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

Detailed Description Text - DETX (52):

Transformation Of Plasmids Into **Bacillus** subtills, Expression And Purification of **Mutant .alpha.-Amylase**

Detailed Description Text - DETX (53):

.alpha.-Amylase was expressed in **Bacillus** subtills after transformation with the plasmids described in Examples 1-3. pHP13 is a plasmid able to replicate in E. coil and in **Bacillus** subtills. Plasmids containing different **variants** were constructed using E. coil strain MM294, the plasmids isolated and then transformed into **Bacillus** subtills as described in Anagnostopoulos et al., J.

Bacter., vol. 81, pp. 741-746 (1961). The **Bacillus** strain had been deleted for two proteases (.DELTA.apr, .DELTA.npr) (see e.g., Ferrari et al., U.S. Pat. No. 5,264,366) and for amylase (.DELTA.amyE) (see e.g., Stahl et al., J. Bacter., vol. 158, pp. 411-418 (1984)). The **bacillus** strain expressing M15L/N188Y was found to form larger zones of clearing than the strain expressing M15L on agar plates containing 1% insoluble starch indicating increased amylolytic activity. After transformation, the sacU(Hy) mutation (Henner et al., J. Bacter., vol., 170, pp. 296-300 (1988)) was introduced by PBS-1 mediated transduction (Hoch, J. Bact., vol. 154, pp. 1513-1515 (1983)).

Detailed Description Text - DETX (82):

.alpha.-Amylase comprising the substitution M15T/N188S made as per Examples 1-4 was compared with wild type **.alpha.-amylase** derived from **Bacillus** licheniformis (Spezyme.RTM. AA20, available commercially from Genencor International, Inc.) in liquefaction at 105.5.degree. C. As shown in Table 2, the **mutant** enzymes provided significantly increased performance in jet-liquefaction of starch, especially at low pH. Pilot scale liquefaction was performed with a primary stage liquefaction at 105.5.degree. C. and a secondary stage liquefaction at 95.degree. C. Amylase was added at 12 LU/g of carbohydrate (dry basis).

Detailed Description Text - DETX (85):

.alpha.-Amylase comprising substitution M15T/N188S made as per Examples 1-4 was compared with wild type **.alpha.-amylase** derived from **Bacillus** lichenformis (Spezyme.RTM. AA20, available commercially from Genencor International, Inc.) in liquefaction at 107.degree. C. As shown in Table 3, the **mutant** enzymes provided significantly increased performance in jet-liquefaction of starch especially at low pH, as shown by the DE value, during liquefaction processes. Pilot scale liquefaction was performed with a primary stage liquefaction at 107.degree. C. and a secondary stage liquefaction at 95.degree. C. Amylase was added at 12 LU/g or carbohydrate (dry basis).

US-PAT-NO: 5731280

DOCUMENT-IDENTIFIER: US 5731280 A

TITLE: Recombinant lipase and alpha-amylase variants

DATE-ISSUED: March 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nielsen; Egon	Copenhagen	N/A	N/A	DK
Rasmussen; Grethe	Copenhagen	N/A	N/A	DK
Halkier; Torben	Frederiksberg	N/A	N/A	DK

APPL-NO: 08/ 448540

DATE FILED: June 15, 1995

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/DK93/00441 filed Dec. 22, 1993, which is incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	1542/92	December 23, 1993

PCT-DATA:
APPL-NO: PCT/DK93/00441
DATE-FILED: December 22, 1993
PUB-NO: WO94/14951
PUB-DATE: Jul 7, 1994
371-DATE: Jun 15, 1995
102(E)-DATE:Jun 15, 1995

US-CL-CURRENT: 510/392, 435/198 , 435/200 , 435/201 , 435/202 , 435/204 , 435/205 , 510/530

ABSTRACT:

The present invention relates to lipase and .alpha.-amylase variants, stabilized towards the inactiviation caused by peroxidase systems, in which variants a naturally occurring tryosine residue has been deleted, substituted with a different amino acid residue at one or more positions. The invention also relates to a method of stabilizing a lipase or an .alpha.-amylase towards the inactivation caused by the preoxidase systems, and detergent compositions comprising a lipase and/or .alpha.-amylase variant of the invention.

13 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Detailed Description Text - DETX (3):
In the context of this invention, a stabilized lipase or .alpha.-amylase **variant is a lipase or an .alpha.-amylase having improved stability** towards inactivation caused by peroxidase systems, when compared to the parent lipase or .alpha.-amylase.

Detailed Description Text - DETX (33):
In a preferred embodiment, the **.alpha.-amylase variant** of the invention is obtainable from a strain of **Bacillus** or a strain of Aspergillus.

Claims Text - CLTX (1):
1. A Humicola, Pseudomonna, Fusarium, Rhizomucor, or Candida lipase or a **Bacillus** or Aspergillus **alpha-amylase variant having an improved stability** towards inactivation caused by a peroxidase system, said peroxidase system comprising a peroxidase or a compound exhibiting peroxidase activity, a source of hydrogen peroxide and a peroxidase enhancing agent, as compared to a parent lipase or **alpha-amylase,** in which one or more naturally occurring tyrosine residues in said lipase or **alpha-amylase variant** has been substituted with an amino acid residue selected from the consisting of phenylalanine, leucine, isoleucine, valine, glutamine, asparagine, serine, threonine, glutamic acid, and histidine.

Claims Text - CLTX (5):
5. A method of stabilizing a Humicola, Pseudomenus, Fusarium, Rhizomucor, or Candida lipase **variant or Bacillus** or Aspergillus **alpha-amylase variant** toward peroxidase inactivation as compared to a parental lipase or **alpha-amylase** comprising substituting one or more naturally occurring tyrosine residues in said lipase or **alpha-amylase variant** with an amino acid residue selected from the group consisting of phenylalanine, leucine, isoleucine, valine, glutamine, asparagine, serine, threonine, glutamic acid, and histidine.

US-PAT-NO: 5652127

DOCUMENT-IDENTIFIER: US 5652127 A

TITLE: Method for liquefying starch

DATE-ISSUED: July 29, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 459984

DATE FILED: June 2, 1995

US-CL-CURRENT: 435/99, 435/201 , 435/202 , 435/203 , 435/204 , 435/205 , 435/275 , 435/72

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of adding a sodium composition to the starch prior to or simultaneously with liquefying the starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Preferred sodium compositions comprise sodium chloride, sodium bicarbonate, sodium benzoate, sodium sulfate, sodium bisulfite, sodium ascorbate, sodium acetate, sodium nitrate, sodium tartrate, sodium tetraborate, sodium propionate, sodium citrate, sodium succinate, monosodium glutamate, trisodium citrate, sodium phosphate or a mixture thereof.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (15):

In PCT Publication No. WO 94/02597, a **mutant .alpha.-amylase having improved oxidative stability** is described wherein one or more methionines are replaced by any amino acid except cysteine or methionine.

Brief Summary Text - BSTX (16):

In PCT publication No. 94/18314; a **mutant-amylase having improved xidative stability** is described wherein one or more of the methionine, tryptophan,

cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (31):

"**.alpha.-Amylase**" means an enzymatic activity which cleaves or hydrolyzes the .alpha.(1-4) glycosidic bond, e.g., that in starch, amylopectin or amylose polymers. Suitable **.alpha.-amylases** are the naturally occurring **.alpha.-amylases** as well as recombinant or **mutant** amylases which are useful in liquefaction of starch. Preferred amylases in the present invention are **.alpha.-amylases** derived from **Bacillus, and particularly Bacillus** licheniformis, **Bacillus** amyloliquefaciens or **Bacillus** stearothermophilus.

US-PAT-NO: 5364782

DOCUMENT-IDENTIFIER: US 5364782 A

TITLE: Mutant microbial .alpha.-amylases with increased thermal, acid and/or alkaline stability

DATE-ISSUED: November 15, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Quax; Wilhelmus J.	Voorschoten	N/A	N/A	NL
Laroche; Yves	Brussels	N/A	N/A	BE
Vollebregt; Adrianus W. H.	Naaldwijk	N/A	N/A	NL
Stanssens; Patrick	St. Denijs Westrem	N/A	N/A	BE
Lauwereys; Marc	Haaltert	N/A	N/A	BE

APPL-NO: 07/ 623953

DATE FILED: November 29, 1990

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
EP	89201735	June 29, 1989

PCT-DATA:
APPL-NO: PCT/EP90/01042
DATE-FILED: June 27, 1990
PUB-NO: WO91/00353
PUB-DATE: Jan 10, 1991
371-DATE: Dec 2, 1990
102(E)-DATE:Dec 2, 1990

US-CL-CURRENT: 435/202, 435/252.3 , 435/263 , 435/275 , 435/320.1 , 536/23.2

ABSTRACT:

Thermostable and acid stable .alpha.-amylases are provided as expression products of genetically engineered .alpha.-amylase genes isolated from microorganisms, preferably belonging to the class of Bacilli. Both chemical and enzymatic mutagenesis methods are e.g. the bisulphite method and enzymatic misincorporation on gapped heteroduplex DNA. The mutant .alpha.-amylases have superior properties, e.g. improved thermostability over a broad pH range, for industrial application in starch processing and textile desizing.

6 Claims, 15 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

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Brief Summary Text - BSTX (16):
EP-A-0285123 discloses a method for complete mutagenesis of nucleic acid sequences. As an example mutagenesis of the B. stearothermophilus .alpha.-amylase is described. Although there is a suggestion that this method can be used to obtain B. stearothermophilus .alpha.-amylase **mutants with improved stability** no examples are given.

Detailed Description Text - DETX (4):
With "improved acid (or alkaline) **stability" we mean that the mutant** enzyme performs better at lower (or higher) pH values then the wild-type enzyme from which it was derived.

Detailed Description Text - DETX (14):
Suitable host strains for production of **mutant .alpha.-amylases** include transformable microorganisms in which the expression of **.alpha.-amylase** can be achieved. Specifically host strains of the same species or genus from which the **.alpha.-amylase** is derived, are suited, such as a **Bacillus** strain. Preferably an **.alpha.-amylase** negative **Bacillus** strain is used more preferably an **.alpha.-amylase** and protease negative **Bacillus** strain.

Detailed Description Text - DETX (132):
Mutant D7 was also found positive in the thermo-stability screening assay (Example 5).

Detailed Description Text - DETX (138):
These molecules were transformed into E. coli WK 6 Mut S and plasmid pools were recovered. These plasmid pools were subsequently transformed into . E. coli WK 6 and the colonies were selected on chloramphenicol (50 .mu.g/ml) containing agar plates. Resulting **mutants were screened for stability** of .alpha.-amylase as described in Example 5.

Detailed Description Text - DETX (170):
a) With the aid of the unique restriction sites within the **.alpha.-amylase** gene (FIG. 4), fragments carrying mutations were isolated from pMaTLia6 **mutants** and subcloned into the homologous position of pBMa6.Lia6. The latter plasmid, which can be replicated either in E. coli or in **Bacillus,** was subsequently digested with SacI and recircularized with T4 DNA ligase. After transformation into **Bacillus** subtilis 1A40 high level **.alpha.-amylase** production under control of the SPO.sub.2 promoter was obtained. Recircularized pBMa6.Lia6 is named pB6.Lia6 to indicate the removal of the E. coli portion of the vector.

Detailed Description Text - DETX (172):

The mutations of **mutants** D7 and 2D5 were transferred to pBMa6.Lia6 by method a) by exchanging the SacII-SalI fragments and **.alpha.-amylase** was recovered from the medium of transformed **Bacillus** subtilis 1A40. Supernatants of both **mutants** were subjected to the screening procedures of Examples and it was confirmed that both **mutants** produce **.alpha.-amylase** which is more acid stable and more thermostable than **.alpha.-amylase** produced by wild-type pB6.Lia6.

Detailed Description Text - DETX (174):

Ultimately pB6.Lia6 **mutants** have been transformed into **Bacillus** licheniformis T9, which is a protease negative, **.alpha.-amylase** negative derivative of **Bacillus** licheniformis T5, (EP-0253455, CBS 470.83). Host T9 has been used to produce high level amounts of **.alpha.-amylase mutants** in a homologous system. The removal of the chromosomal **.alpha.-amylase** gene renders this strain very suited for the production of **mutant .alpha.-amylase** as no contaminating wild-type **.alpha.-amylase** is being produced anymore. Enzyme recovered from this strain has been used for industrial application testing. The industrial use of **mutants** pB6.Lia6.2D5 and pB6.Lia6.D7 was demonstrated.

Detailed Description Text - DETX (204):

To test the industrial application of alkaline .alpha.-amylase **mutants a test is performed on the stability** at 20.degree. C. in the following solution:

Claims Text - CLTX (1):

1. An isolated **mutant .alpha.-amylase wherein said mutant .alpha.-amylase** has a replacement of at least one amino acid in a corresponding wild-type **.alpha.-amylase** obtainable from **Bacillus** licheniformis and wherein said **mutant .alpha.-amylase** exhibits one or more improved properties relative to the wild-type **.alpha.-amylase** selected from the group consisting of improved thermostability, improved stability at a pH below 6.5, improved stability at a pH above 7.5, and improved acid stability as a result of said replacement, wherein said replacement is one or more amino acid replacements selected from the group consisting of Ala-111-Thr, His-133-Tyr and Thr-149-Ile.

US-PAT-NO: 4806426

DOCUMENT-IDENTIFIER: US 4806426 A

TITLE: Mutant microorganisms containing recombinant DNA and their use in the production of amylases

DATE-ISSUED: February 21, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Colson; Charles A.	Dion-Valmont	N/A	N/A	BE
Lejeune; Philippe	Verviers	N/A	N/A	BE
Walon; Corinne	Wavre	N/A	N/A	BE
Willemot; Karine	Charleroi	N/A	N/A	BE

APPL-NO: 06/ 737311

DATE FILED: May 23, 1985

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	8414271	June 5, 1984

US-CL-CURRENT: 435/252.31, 435/202 , 435/839

ABSTRACT:

Recombinant DNA containing amylase-coding genes is prepared by cleaving DNA from various donor microorganisms and combining portions of the DNA with the plasmid pUB110. Strains of E. coli or B. subtilis containing the recombinant DNA are grown in fermentation media to produce the amylase enzymes.

2 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Brief Summary Text - BSTX (7):

We have now developed recombinant DNA which comprises certain amylase-coding genes described in U.S. Pat. No. 4,469,791, but which is derived from a plasmid not specifically described in that patent. The plasmid is pUB110, whih was also described in an article in the Journal of Bacteriology 1978, Vol. 134, pp. 318-329. The plasmid pUB110 comprises a gene coding for resistance to kanamycin or to analogous antibiotics inactivated by the nucleotidyl

transferase enzyme. We have found that the recombinant DNA derived from this plasmid and certain amylase-coding genes may be introduced into a host-microorganism and that, particularly when the host is B. subtilis, **mutant** strains may be produced and cultivated which have enhanced stability and high copy numbers. The **mutant** microorganisms which comprise the novel recombinant DNA may be used, therefore, on an industrial basis for the production of amylase and in particular, for the production of the **alpha-amylase** of B. megaterium (**Bacillus** megaterium), an amylase possessing particularly useful commercial properties.

Detailed Description Text - DETX (9):

These three strains may be mutated by the process described for the analogous BAS35 to give kanamycin-resistant **mutants possessing increased stability** and high copy numbers.

Detailed Description Text - DETX (13):

B. subtilis BGSC 1A289: The organism is a **mutant** deficient in the gene coding for **alpha-amylase**. It was obtained from the **Bacillus** Genetic Stock Centre of Ohio State University.